

Neuronal and cognitive effects of estrogens

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Interview with Bruce McEwen

Q: *Estrogens are called "sex hormones" and their primary function is usually viewed as a reproductive one. When did it first come to our attention that they may also have an effect on the nervous system?*

A: Actually the first experiment in the history of endocrinology deals with hormone effects on the brain, although the person who did the experiment did not really do it for that reason. A German professor named Berthold, who worked in Göttingen, published a paper in 1849 called "Transplantation of the testes of the domestic rooster". What he did was to take advantage of the fact that when you castrate a rooster, the characteristic mating behavior and crowing of the rooster disappear and of course, the secondary sex characteristics disappear. And then he transplanted the testes into the abdominal cavity of these capons and found that over time the secondary sex characteristics and the mating behavior returned. When he autopsied the testes and discovered that they were not innervated but were highly vascularized, he thought that there must be a blood-borne substance, that we now know as testosterone, and he concluded that it must affect the brain

in some way, directly or indirectly.

The second step in this process took place in the 1960s and 1970s, by which time we had had crystalline and pure steroid hormones for many decades. Using the rooster model again, a scientist named Ron Barfield put crystals of testosterone into the hypothalamus of capons and found that he could bring back the sexual behavior and the crowing of the capon just as Berthold had done with transplanted testes. Similar studies were done about the same time with estrogens in the female rat brain showing that you could restore sexual behavior that was lost after ovariectomy. About 1960, tritium-labelled estrogens were synthesized and the tritium provided much higher specific radioactivity that allowed the identification of steroid receptors by their binding properties. The first studies were done in the uterus and resulted in the identification of binding sites, receptors that bind to DNA. Then studies by my colleague, Don Pfaff, and others, notably Walter Stumpf, used steroid autoradiography in the brain to show that there were certain nerve cells that would take up tritium-labeled estradiol and retain it in the cell nucleus, which is where receptors for estrogens are located in the cell. This is about the time we began working in this area. Don, and others, mapped steroid receptors in the brain. Initially, the most striking observation was that the receptors were located in the hypothalamus, in the amygdala, in the areas that were

traditionally associated with reproductive behavior. But sparse estrogen sensitive cells were also observed in other parts of the brain, for example in the hippocampus, the midbrain, and the brainstem. They were so few and far between that they were generally ignored at that time. Many years later in my own lab, we went back to the hippocampus and confirmed that there were scattered estrogen-sensitive neurons. And then a decade later – these things don't happen fast – a then graduate student, who is at this meeting and who is now a faculty member at Northwestern University in Evanston, Illinois, Catherine Woolley, investigated the actions of estrogens in the hippocampus and discovered that they regulate the formation of new synaptic connections between nerve cells. Now, she did this experiment because we already knew that in the hypothalamus estrogens cause new synapses to form and that is probably part of the process by which they help to turn on sexual behavior in a female rat. But here in the hippocampus, they were doing the same thing. We knew the hippocampus becomes more excitable when estrogen concentrations are high. You can elicit seizures more easily due to these kinds of excitatory synapses that are being made, so the connection then was made with synapse formation and increased excitability and probably increased synaptic transmission. In parallel, there were other studies going on. Dr. Victoria Luine, who is also at this meeting, was a postdoc in my laboratory in the 1970s, and about 1980 she discovered that estrogens regulate

cholinergic function. They induce the formation of cholinacetyl transferase in the basal forebrain. And this is again a part of the brain that was not part of the traditional area for the control of reproductive behavior. Similar observations were made by another group with regard to the catecholaminergic neurons in the brainstem. Walter Stumpf's laboratory in Chicago and now in North Carolina showed that some of the catecholaminergic neurons of the brainstem have estrogen receptors and Allan Herbison at this meeting gave a beautiful paper showing that there are in fact clusters of estrogen receptors in catecholaminergic neurons, that is A1, A2, and A6. A1 and A2 have estrogen receptors but only in the ventral part, not in the dorsal part, and A6 which is the so-called locus ceruleus does not appear to have any. Cindy Bethea at this meeting told us about estrogen receptors and regulation of the serotonin system. Jill Becker talked about regulation of the dopaminergic system. In the dopaminergic system, unlike the other systems, there has been no evidence of any intracellular estrogen receptors. So estrogens appear to have effects in many parts of the nervous system.

Q: *What are some well-documented effects of estrogens on neurological and cognitive function ?*

A: Well, there is the regulation of excitability of seizure thresholds. People who are vulnerable to epilepsy show more

seizures if they have high estrogen levels. There is also an animal model of increased seizure elicitation in female rats primed with estrogens when you stimulate the hippocampus. Estrogens improve motor fluidity, motor coordination, and they act in both the neostriatum and also in the cerebellum. Sheryl Smith, at this meeting, gave an absolutely spectacular example of how, in the olive of the cerebellum, estrogen treatment causes synchronization of the firing pattern of nerve cells, and she thinks that it does so by inducing gap junctions, which are links or openings between cells, so you don't have a synapse but rather a syncitium of cells that are firing together and she thinks that estrogens may be producing an effect in that way. This is a totally unexpected finding which is getting away from the traditional idea that one has to look for effects only where the estrogen receptors are found. People have now begun to find effects where there are very few, if any, estrogen receptors.

Another cognitive effect of estrogens was discussed at this meeting by Barbara Sherwin who talked about the improvement of verbal memory, which is a specific hippocampal-dependent memory in women. The best example of this is her study of women treated with GNRH agonists to suppress uterine fibroids, who complained of memory deficits. When these women were given estrogens while still on the GNRH, their memory improved, and then it worsened again when the estrogens were withdrawn. This is a very nice example of

reversible effects that point to the hippocampus as a particularly important target and that is where we have seen the synapse formation. So we tend to link these things together. This synapse formation has captivated neurobiologists who are interested in the neurophysiology of learning and memory and who are not particularly interested in hormones, but having hormones that modulate synapse formation is really quite a novel **concept**.

Q: We now know that there is more than one form of the estrogen receptor. What do we know about the functions of the alpha receptor and the more recently described beta receptor ?

A: In the beginning there was, and still is, steroid autoradiography which measures the presence of high affinity estrogen binding sites in the cell nucleus. We know now that there is more than one form of the estrogen receptor – there is the alpha form which is the one that was recognized first and cloned, and now there is the beta estrogen receptor. The lab of Dr. Gustafsson, who is at this meeting, is one of the major labs that has identified the beta receptor. The beta form actually has several subforms and even the alpha form may have several subforms, the functions of which are not entirely clear. So, the main distinction is between the alpha receptors and the beta receptors. They are very similar in many of their actions and in their affinity for estrogen, but there are differences in specificity that

allow for the development of selective agonists and antagonists, and they work differently when you have nonsteroidal antiestrogens like tamoxifen or raloxifene, which are sometimes called "SERMs" or selective estrogen response modulators. SERMs will block the actions of ER alpha and ER beta when these receptors are working through the DNA binding domain, the estrogen response element that has been the traditional site of estrogen action. But then Peter Kushner and others have called attention to the fact that the same receptors also work on what is called an AP-1 site. It is a different DNA sequence, and here, they do not work by binding directly to that site, they work by binding to another protein which is bound to yet another protein and that protein binds directly to DNA. It is sometimes called tethering. And here, ER alpha and ER beta work quite differently, and that again depends on the ligand. The SERMs like tamoxifen and raloxifene work in different ways from estradiol itself. Having said that, a new finding at this meeting by Elis Levin is that these receptors, both ER alpha and ER beta, of which a tiny percentage can be captured by machinery inside the cell, can apparently be modified in some way on their surface perhaps by attaching lipids or something, and inserted at or near the cell membrane. Now, these receptors would have escaped detection by traditional autoradiography and also by immunocytochemistry which looked for the receptors in the nucleus. People always see a haze in the cytoplasm but they tend to think of it as a

nonspecific signal, but it may not be. Levin's experiments were done in a transfection system. He took cells, he put the estrogen receptor messenger RNA into the cells and then he found the receptors expressed in a form that is able to regulate second messenger systems. We don't know yet if normal cells expressing the receptor also have this location out in the periphery, but it is very likely that they will and then we have the situation that was described at the meeting – but heretofore without a basis in cell biology – that estrogens also produce effects on membrane-related events, such as their ability to regulate a variety of second messenger systems. Not only that, they stimulate electrical activity of cells, and this has been known for years. The only way these effects were distinguished from the genomic effects is that they were rapid and they tended to die away after the estrogen was withdrawn, and so people loosely talked about membrane receptors. So, we have now a situation in which traditional estrogen receptors exist not only in the cell nucleus but also in other parts of the cell, for example, near the cell surface where they can couple to second messenger systems. Both Dr. Toran-Allerand and Dr. Levin talked extensively about their work showing that you could activate a second messenger pathway with estrogens working through either ER alpha or ER beta. So, now if you go back to parts of the brain where we have never seen these ER alpha and ER beta at the cell nuclear level, it is possible then that there could be these other sort of

cryptic forms of the receptor in or near the cell membrane where they would have escaped detection and where they are doing something quite different. **One major** consequence of **second messenger** signalling is to activate genes. Thus besides their direct actions in the nucleus, estrogens have an indirect way to activate genes. This is even more indirect than when nuclear estrogen receptors act through the AP-1 response element where the receptor binds to AP-1 proteins and not directly to DNA. I think that this meeting was particularly because the participants were ready to put together the phenomenology of diverse estrogen actions in very important systems of the brain with the multiple cellular mechanisms by which estrogens can produce their effects on brain cells. The meeting established a new agenda of research for the next several decades.

Q: *Given the differences in the estrogen levels between males and females on the one hand and between pre and postmenopausal women on the other hand, if estrogens are having an effect on neurological function, you might expect to see differences in neurological functions between men and women, and between premenopausal and postmenopausal women? Is that the case?*

A: Yes, it is. But we have to consider several issues here. Around the menopause, there is a withdrawal of estrogens but this is not a complete withdrawal. Also, some woman who have body fat can manufacture estrogens from their own adrenal

androgens. In men, testosterone levels tend to decline much more gradually as they age. And then there is the whole other aspect of developmentally programmed sex differences. Even if you gave the female testosterone or gave the male estradiol, they wouldn't produce the same effect because the brain has been programmed during early development to do something different in men and women. Examples of these differences would include differences in pain sensitivity.

Women have different pain pathways than men, and these seem to be developmentally programmed in addition to being affected by gonadal hormones. Women tend to have more depressive illness, while men have more antisocial behavior and substance abuse - thus men and women express their emotional problems in different ways. Then there are gender differences in the onset and frequency of dementia which, according to some data from this meeting, are also related perhaps to genotypes like the APO E4 genotype. This is a very complicated story and is still not yet clear. In addition, the male and female brain are each programmed early in life, and some systems, the noradrenergic system, the cholinergic system, the basal forebrain and the hippocampus may be programmed differently in males and females. Gonadal hormones are not going to work in the same way men and women. When you add to that the hormonal differences that exist between the sexes and the changes that take place over the menopause then you see that men and woman will have different patterns of disease and

vulnerability. I think it's important to add that this is part of the very important rationale for a gender-based approach to drug treatment and this has become much more popular now, particularly in the United States, but also in Western Europe. When you think about it, drugs have been on the whole developed in men, tested in men, and then given to women, but with very little data until it became apparent what did and didn't work. Now, we are beginning to develop drugs from the beginning with some realization of how they may work differently in men and women.

Q: *Nature provides us with physiological situations where there are great fluctuations in hormone levels - the menstrual cycle and pregnancy, Are there also fluctuations in neurological function in these circumstances ?*

A: Yes, one of the best examples is catamenial epilepsy. This is a disorder that occurs only in some women but tends to be worse in the follicular phase of the cycle when estrogen levels are high and progesterone levels are low. When progesterone levels go up, the symptoms and risk of seizures tends to go down. During pregnancy when progesterone levels are high, the risk is very low. Similarly, although it is probably not explained by the same mechanisms, the symptoms of premenstrual syndrome, the extreme mood variations that go on in a vulnerable subgroup of women, vary according to the follicular and luteal phase of the cycle, and if you stop the cycle in some way, for example with a birth control

pill or a GnRH agonist, the mood changes disappear. But I am told that they tend to stop at some level that may not be an optimal level for the woman in question. She may be in a terrible mood all the time rather than in a good mood some of the time. There are other examples, but those are two good examples.

Q: *Let's get on to therapeutic potential of estrogens in neurological disease and I want to focus on hormone replacement therapy. Is there any evidence that hormone replacement therapy affects the incidence of neurological disease and particularly dementia and Alzheimer's disease ?*

A: The evidence is quite strong that it does have a protective effect, but there is not much evidence that it has any effect when Alzheimer's disease is already well-established. The qualification though is that even the longest treatment studies have not gone on long enough to really see if there is an effect say over a longer period, such as five to ten years. Under these circumstances, there might be a beneficial effect, but I think most people would put their bets on a protective effect related to starting estrogen replacement during the menopause. Now, what does protection entail? There are many different levels. If you take these systems of the brain that I have been talking about, cholinergic, adrenergic, hippocampus, serotonergic, cerebellum and you remove estrogens, reversible changes occur. Peoples' memories fail in certain ways, they become

less coordinated, their moods may become worse, they may become depressed. If you give back estrogens you can reverse this. There were some interesting observations mentioned at the meeting about how tacrine, the cholinergic enhancer, seems to work best with estrogens present and how the antidepressant fluoxetine seems to work best when estrogens are present. These are examples again of how they potentiate normal functions. These functions are lost in women who have profound estrogen withdrawal and again we have to remember that women with more body fat may actually make more of their own estrogens, so they may not need any supplemental estrogens. But in those women who are profoundly deprived of estrogens, their functions are going to be worse and we don't know exactly what is happening within their nerve cells. The brain is remarkable in its ability to compensate and you heard some of the discussion this morning about critical periods. People wonder whether there is a period of time during the perimenopause when, as estrogen levels begin to decline, replacing estrogen would actually help in some fashion. This is sort of a hunch that people have, but there is no hard data.

Now, when these systems begin to function less well, at the same time the effects of ageing continue of course there is some degree of increased oxidative load, like on the cardiovascular system and on the brain, and here we get into another dimension of estrogen action potential. One possible effect might be suppressing the formation of the

toxic beta-amyloid protein and Sam Gandy talked about that.

At the same time, estrogens seem to enhance production of the naturally secreted form of the amyloid precursor protein, which may be beneficial for synaptic function and may actually be protective. Another potential effect that both Jim Simpkins and Christian Behl from Germany talked about involves the oxidative aspects and there we get into a whole new domain. On the one hand, they have evidence that there may be actions of estrogens that don't involve traditional receptors, because they can create molecules that have no interaction whatsoever with the known intracellular estrogen receptors and yet in the vitro system they have some neuroprotective properties that reduce free radical formation. But there is also data that the traditional estrogen receptors, ER alpha and perhaps even more so ER beta, may be interacting with some of these signaling cascades and may be partly responsible for the beta regulation as I mentioned but that's not clear. The mechanism and the receptor involved in Gandy's study is not known at all. The safe conclusion for the moment, I think, is that there seem to be both traditional receptor-mediated neuroprotective steps and also possibly unique antioxidant actions where it might be possible to design estrogens that bypass all the known receptors and have some protective antioxidant properties. There, the big question that no one can really answer clearly is: "So what's the advantage of estrogens as antioxidants as opposed to having vitamin E or

ascorbic acid?" And I think the best answer is what Behl said and that is that vitamin E doesn't get into the brain very well, because it's a large molecule and it may just get to the vascular bed. And Simpkins had a model which indicated that estrogens are catalytic but they can be oxidized and reduced, and that this ability is part of a catalytic event that helps to regenerate glutathione which is a much more prevalent reducing agent. And so Simpkin's model explains why estrogens would be particularly advantageous over other kinds of reducing agents, things like vitamin E, because of that catalytic role and also because they get into the brain better.

Q: *What about Parkinson's disease? I read in paper that you wrote in 1994 that high levels of estrogen may actually exacerbate symptoms of Parkinson's disease.*

A: Well, that's an interesting point and this is where Jill Becker's work has been so important. Back in the old days when women were first given contraceptive pills, they were ten times or more higher than the doses that are given now. The first reports in the neurological literature were that high doses of estrogens would actually exacerbate symptoms of Parkinson disease which led to the view that estrogens were antidopaminergic. And indeed if you give male or female rats high doses of estradiol you can actually cause an inhibition of dopamine-dependent events. But Becker has

come along and consistently shown that if you ovariectomize female rats and give them back low dose estrogens, they actually enhance dopaminergic function. So the loss of motor coordination in a postmenopausal woman which may result in her having slower reaction times when she is driving a car or cause her to lose her balance and fall, and break her hip or wrist, these are events that can actually be reversed by low-dose estrogen therapy. In other words estrogens have a biphasic effect on dopaminergic activity.

Q: *Regarding the therapeutic effects of estrogens, more more women these days are on long term tamoxifen for breast cancer. Are we seeing any sort of negative estrogen deprivation effect in these women ?*

A: Now, this is a question that was raised repeatedly throughout the meeting and I think there was generally a lot of concern about the consequences of tamoxifen. There have been very few systematic studies of tamoxifen or the new compound raloxifene, but there are anecdotal reports that everyone seems to agree upon. In the first place, if you give it to women perimenopausally or right after the menopause, it tends to cause hot flushes. So, it tends to do what the withdrawal of estrogens would do because it's acting as an antagonist. A pretty well-documented finding, not involving the brain, is that if you given women tamoxifen when they are perimenopausal and still have

estrogens of their own, it accelerates bone mineral loss. And this is what you would expect of a partial agonist which is also an antagonist. If you wait until the woman is completely withdrawn from her own estrogens and you give tamoxifen, then you see the agonist effect. It helps to protect bone. Now, one of the key questions relates to the critical period argument. People wonder whether tamoxifen or raloxifene, if given at the wrong time, like during the perimenopausal period, might actually increase the incidence of cognitive deficits and even the risk for Alzheimer's disease. We have no evidence, not even anecdotal evidence, but it is a theoretical possibility, and that's why the critical period issue that we talked about is so important.

There is again anecdotal data that tamoxifen does apparently cause some woman to have worse cognitive function but again it's only anecdotal and it has never been studied in a systematic way, or at least such studies, if they exist, have not yet been published. I think the bottom line is that there are many actions of estrogens in the brain, and each one makes a contribution to many functions whether it is mood or cognition. Furthermore, each one may involve different estrogen receptors, different response elements or even membrane versus other kinds of receptors. So the jury is out regarding where in the nervous system the SERMs are going to be working and whether they are going to work as agonists or antagonists. Thus, you are probably going to get a mixed bag of effects no matter what. And it may be

theoretically impossible to design a compound like a SERM that does everything you want it to do and does none of the things you don't want it to do. So you are back to using estradiol itself with all of the inherent problems there. This is a very open question, and I think it's the area where there was the most concern and agreement that we just don't know enough.

Q: We have touched on a number of issues discussed at this meeting but let me ask you what struck you most. What were the most promising things reported ?

A: Well, I think that at the molecular and mechanistic level there is good evidence now for other modes of estrogen action both in terms of receptors near the membrane as well as in the nucleus involving also the activation of second messenger systems as well as nongenomic actions and neuroprotective actions. There is now better evidence for this than ever before and a greater open-mindedness on the part of scientists to consider all these mechanisms and to envision that there is more than one mechanism that's likely to be involved in many of these effects. Having been in this field for 30 years, it does strike me that everybody is converging to that point. The second thing is the interest that is being shown in neural systems outside the hypothalamus, that tended to be ignored at the beginning because they did not have as many estrogen receptors. Each

of the stories that we heard had a level of sophistication and intrinsic interest and there are beautiful studies being done. They are of great interest from an endocrinology point of view but also from a basic neurobiology point of view, and they have tremendous implications for all of these diseases we are talking about whether it is cognitive impairment, depression, movement disorders, pain, or neurodegenerative disorders. And then finally I was impressed that the clinicians in the audience, are very much aware of this. Clinical work is being done that is incredibly difficult for the reasons we have been discussing. But, in a concerted effort with pharmaceutical firms, there is really lot of excitement to try to develop new strategies and these strategies may involve cotreatment, like the Tocrine and estrogen or the serotonin reuptake inhibitor (e.g. fluoxetine) and estrogen combination, or the idea that you might want to give a SERM for one set of indications, perhaps a SERM that doesn't get into the brain, and then give an estrogen that gets into the brain for another set of indications. So, I think there needs to be a lot more creative thinking on this topic.

Q: From what you have said it is clear that a lot remains to be done. How would you like to see that the field evolve and what do you think are the important issues that we need to resolve?

A: Well, there are some intrinsic, scientific issues we have

already talked about. It may be impossible to do everything right. We are not going to have a magic bullet, so it's a matter of trade-offs – like weighing the short-term problems with breast cancer against the longer term problems of dementia or bone loss or other things. I think this just entails a lot more interactive discussions between researchers, clinicians and drug companies to develop the best strategies possible. One issue I would like to conclude with is the important role of the pharmaceutical industry. Clearly the pharmaceutical industry has a vested interest in many of these issues. The pharmaceutical industry functions the way it does, because it has to survive and prosper commercially and so it tends to develop and launch products that will find a large market. In the United States, it's the conjugated estrogens from pregnant mare's urine. In Europe, it tends to be more the estrogen skin patch. There are also products containing progestins, medroxyprogesterone acetate, which some people feel may be undoing most of the good things that estrogens do, and there was some data at this meeting on that important point. I don't know how we are going to get around this, because the pharmaceutical industry is the pharmaceutical industry. It works the way it works, it sponsors meetings, it sponsors research, and to do this it must sell marketable products. But this presents a big obstacle from the clinical research point of view. There are so many forms of estrogen replacement therapy out there that it becomes difficult to do any study that results

in a clear-cut conclusion. Some women are getting one product, some are getting another together with progesterone or together with medroxyprogesterone or something else, and they are being treated at different times in their lives with different doses without control of blood levels and there are individual variations. So, the fact that we have come this far and know as much as we know clinically, is in some ways a miracle given this sort of tower of Babel out there. I think clinicians realize this. There was a lot of discussion at the meeting about this point and I am just echoing what people were saying. There has to be some better way of working between the pharmaceutical industry, clinicians and researchers to develop rational strategies and to get rid of strategies when they turn out to be either worthless or even harmful.
