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14. ABSTRACT : Pharmacological tools that are effective in alleviating symptoms of fear, anxiety and depression while enhancing cognition remain to be elucidated. Current strategies of targeting select CNS neurotransmitter systems result in pharmaceutical agents that are either non-selective in their actions and/or take significant amounts of time before they are effective. In contrast, our strategy for the development of anxiolytic agents has been to identify compounds that can selectively target Estrogen Receptor (ER) Beta (β). It is now well established that estrogen receptors are found in near equivalent amounts in the brains of both men and women. While conducting studies in the neurobiological actions of ER β using specific agonists to both receptors (α and β), our laboratories came upon results that have military significance for both women and men. Successful completion of the pre-clinical phase of this multiphased project will provide agents that may be useful for enhancements in the therapeutic and prophylactic management for the alleviation of fear and anxiety-related behaviors, improvements in cognitive functions related to learning and memory, alleviation of depression and depressive mood states, improvements in spatial mapping behavior, improvements in coordination and motor skills, and enhancement of attention and state of readiness. In this proposal, we will characterize the cognitive enhancing and fear suppressing ability of a select group of ER β agonists (steps 1 and 2 of the six steps in this preclinical phase of the work). These include the parent compound and its R- and S-enantiomers. Subtle chemical modifications of these compounds to alter characteristics such as half-life and delivery to the brain may be attempted in future studies to improve pharmacodynamic targeting of the pharmaceutical compounds. The results of these studies strongly support the potential for the development of an advanced medical technology that is pharmacotherapeutically capable to enhance operational readiness in our warfighter as well as to enhance performance in the battlefield. Special Forces and other specialized units in the military, such as the Army Rangers and Navy Seals, routinely are required to perform under extreme conditions, typically lacking sleep or function in isolation as single/double-person units. They are required to conduct multiple sophisticated tasks while maintaining a high level of alertness and cognitive function. In addition, the ability to process sensory information is also taken to a new level.					
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INTRODUCTION

Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

The goal of this project is to design novel compounds that selectively bind estrogen receptor beta ($ER\beta$) to alleviate fear and anxiety-related behaviors and enhance cognitive function. $ER\beta$ is a recently described member of the steroid/thyroid hormone receptor superfamily. Although it was originally cloned and named based on homology to the classic estrogen receptor (α), $ER\beta$ does not appear to be a critical component of reproductive physiology. For example, $ER\beta$ -knockout-mice have few reproductive deficits, in contrast to $ER\alpha$ -knockout mice, which are sterile. Despite its name, $ER\beta$ is not exclusively a receptor for estrogen. $ER\beta$ can bind a number of other hormones and recent studies suggest that in males, $ER\beta$ is the functional receptor for 5α -androstane- 3β , 17β -diol (3β -diol), an endogenous metabolite of the testicular androgen, dihydrotestosterone. $ER\beta$ is also synthesized in brain with a distribution that gives clues to its function: $ER\beta$ is found at high levels in areas that regulate stress responses and cognitive function. Consistent with this, $ER\beta$ knockout mice show increased anxiety-related behaviors and reduced cognitive function. Our preliminary findings, described in the original application, support this possibility. Acute administration of the non-steroidal, $ER\beta$ selective agonist, diarylpropionitrile (DPN) dramatically reduces anxiety related behaviors in female AND male rats when examined in behavioral tests such as the elevated plus maze and open field test. DPN treatment also potentially inhibits the hormonal responses to physical and psychological stressors.

In this proposal, we will test the hypothesis that estrogen receptors may alleviate fear and anxiety-related behaviors as well as to enhance cognitive function. One approach to test the hypothesis that $ER\alpha$ or $ER\beta$ are specifically involved in altering anxiety-related and cognitive behaviors is to use pharmacological agents that work as selective $ER\alpha$ or β agonists or antagonists. Such compounds have recently been described and have been used for the generation of the preliminary data presented in the original application. Meyers et al. (2001) found that DPN is a non-steroidal ER agonist with a 70-fold higher relative binding affinity and 170-fold higher relative potency in transcription assays for $ER\beta$ than with $ER\alpha$. We have synthesized DPN and are in the process of synthesizing the next generation DPN, and have begun to stockpile sufficient amounts necessary for behavioral testing in rats. Although DPN appears to have robust effects on anxiety-related behaviors (see preliminary data), it is possible that more selective agonists may be more effective. DPN possesses a chiral center and as a result, it is synthesized as a racemic mixture containing both the R- and S-enantiomers in equimolar amounts. Molecular modeling studies of the $ER\beta$ ligand binding domain suggest that $ER\beta$ binding of the R- or S-enantiomer are not equivalent (Sun et al., 2003), with S-DPN having a greater binding affinity for $ER\beta$ than R-DPN. Consequently, it has been proposed that selective $ER\beta$ binding activity of the racemic mixture is actually representative of high affinity and selective binding by only one enantiomer (S-) and not the other (R-).

BODY

Our hypothesis states that selective ER β agonists can be used to alleviate anxiety and stress related responses and enhance cognitive function. Furthermore, we predict that this will occur in the absence of any toxic effects. The design and development of such compounds will have high potential for the therapeutic and prophylactic treatment of stress and anxiety in the warfighter, while maintaining state of operational readiness and enhanced cognition.

To test this hypothesis, we proposed the following objectives:

Objective 1. To develop and test 2nd generation agonists with increased selectivity and affinity for ER β .

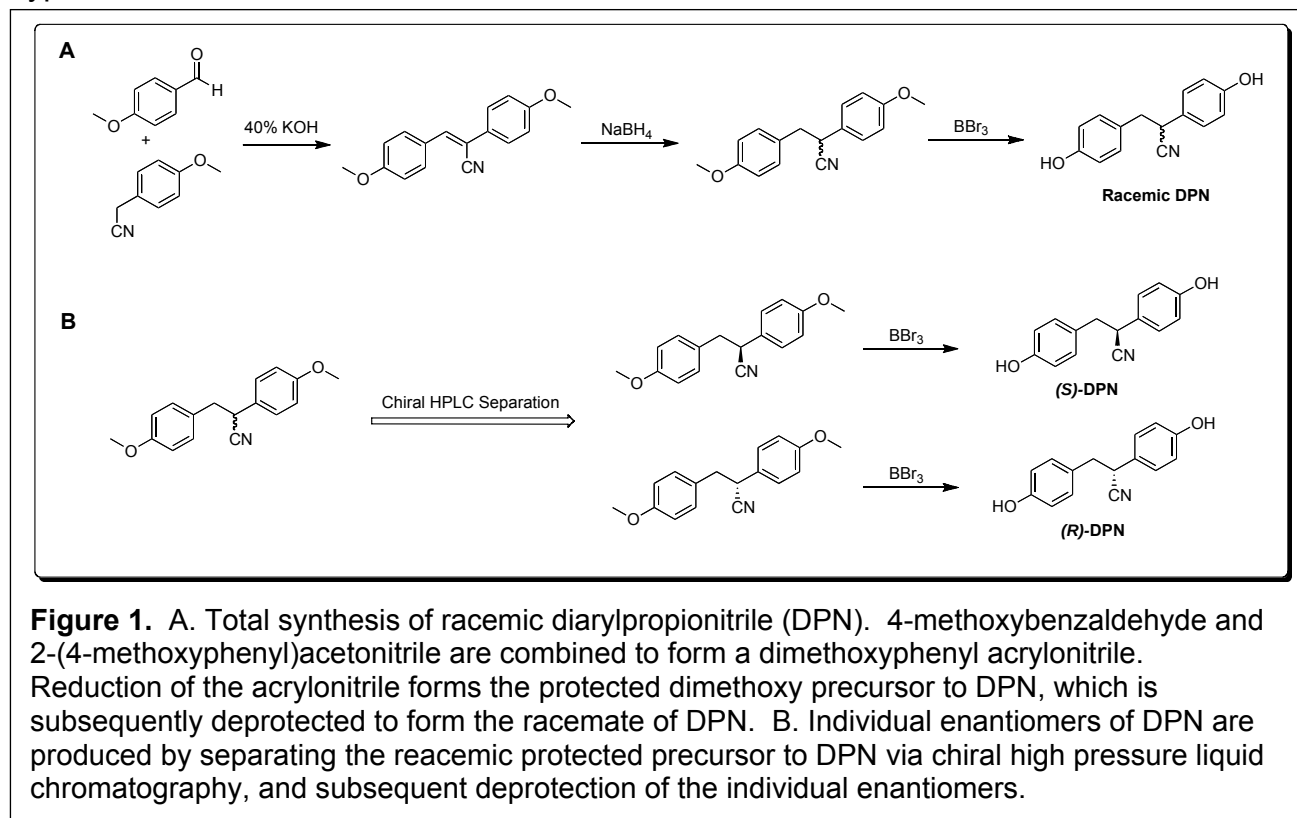
Objective 2. To confirm that DPN and 2nd generation ER β agonists are devoid of toxicologic side effects.

Research Accomplishments:

Objective 1. To develop and test 2nd generation agonists with increased selectivity and affinity for ER β .

Development of Second Generation ERbeta agonist, DPN

Diarylpropionitrile is a selective ERbeta agonist that exists in a racemic mixture. Like all chiral molecules, the R- and S- enantiomers of DPN are mirror images of one another, but they are not superimposable due to its 3 dimensional structure. Molecular modeling studies (Sun et al., 2003) have indicated that the beta ring of the S-enantiomer would mimic the A-ring of 17 β -estradiol and the result would be greater selectivity and tighter binding for ERbeta than the S-enantiomer, or the racemic mixture. We have tested this hypothesis in the studies described below.



Approach:

We have used HPLC with a chiral phase column to separate the –R and –S enantiomers of DPN. The elution of the DPN enantiomers from the column was monitored using mass spectrometry. The enantiomers themselves were verified by recrystallization followed by NMR. The conditions for separation were optimized initially to allow complete separation of the two enantiomers (See Figure 1 above).

The second generation ERbeta agonist has a higher binding affinity to ERbeta than DPN

Approach:

Enantiomers were tested for their ability to bind recombinant ERbeta and ERalpha using standard binding assays with 3H-estradiol as the trace ligand. Competition curves were constructed based on the ability of DPN-S, DPN-R or DPN-racemic to compete with 3H-estradiol for binding the receptor.

Results:

The second generation ERbeta agonist, DPN-S, has a higher affinity to the ERbeta relative to DPN racemic and DPN-R. Even though DPN-S also has a higher affinity to the ERalpha, they still bind at a low affinity relative to their binding to ERbeta (2 orders of magnitude). The calculated Ki values for the enantiomers of DPN for ERalpha and ERbeta are shown in Table 1 below.

Table 1. Calculated Ki Values for DPN binding to recombinant ERalpha and ERbeta.

	ERBeta	ERAlpha
DPN Racemic	2.9 nM	134.6 nM
DPN-S	1.4 nM	70.4 nM
DPN-R	7.6 nM	249.0 nM

These receptor binding curves are shown in Figure 1 (ERbeta) and 2 (ERalpha), below. As shown in figure 1 for ERbeta, when compared with the ability of DPN-racemic to compete for 3H-estradiol binding, DPN-S shifted the curve to the left (tighter binding), whereas DPN-R shifted the curve to the right (weaker binding). These results are in accordance with the hypothesis put forward by Sun et al (2003) based on molecular modeling studies.

As shown in figure 2, DPN-S was also more efficient at displacing 3H-estradiol from ERalpha, compared with DPN-racemic. DPN-R was less efficient. Based on these data, it appears that although the –S enantiomer binds ERbeta with greater affinity, it does not provide an increased selectivity as predicted by the molecular modeling studies.

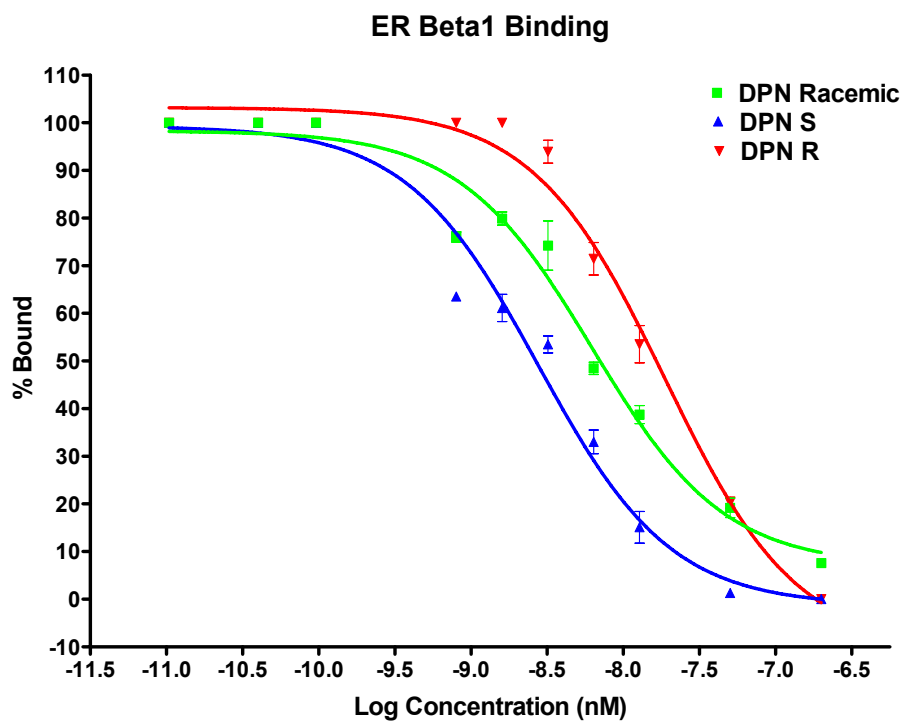


FIGURE 2. DPN-R and DPN-S Binding Studies with ERbeta. The data suggest that DPN-S has a higher affinity to ERbeta compared to the racemic form and DPN-R.

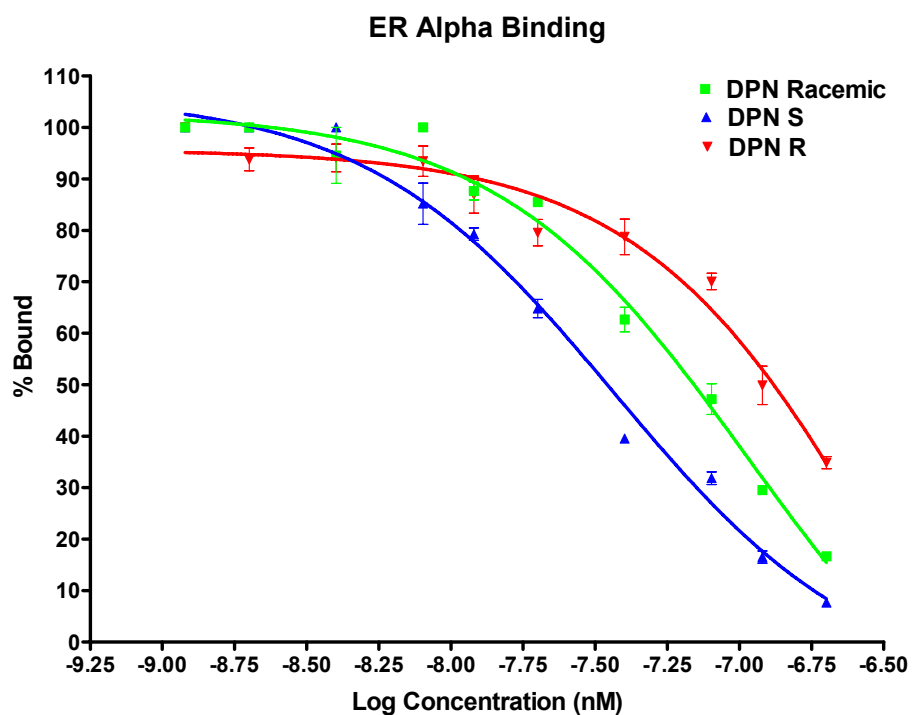


FIGURE 3. DPN-R and DPN-S Binding Studies with ERalpha. DPN-S has a slightly higher affinity to the racemic form and DPN-R but overall, the curves suggest a low affinity binding.

The second generation ERbeta agonist, DPN-S, are more effective than DPN-racemic at inducing transcriptional activity at the ERbeta.

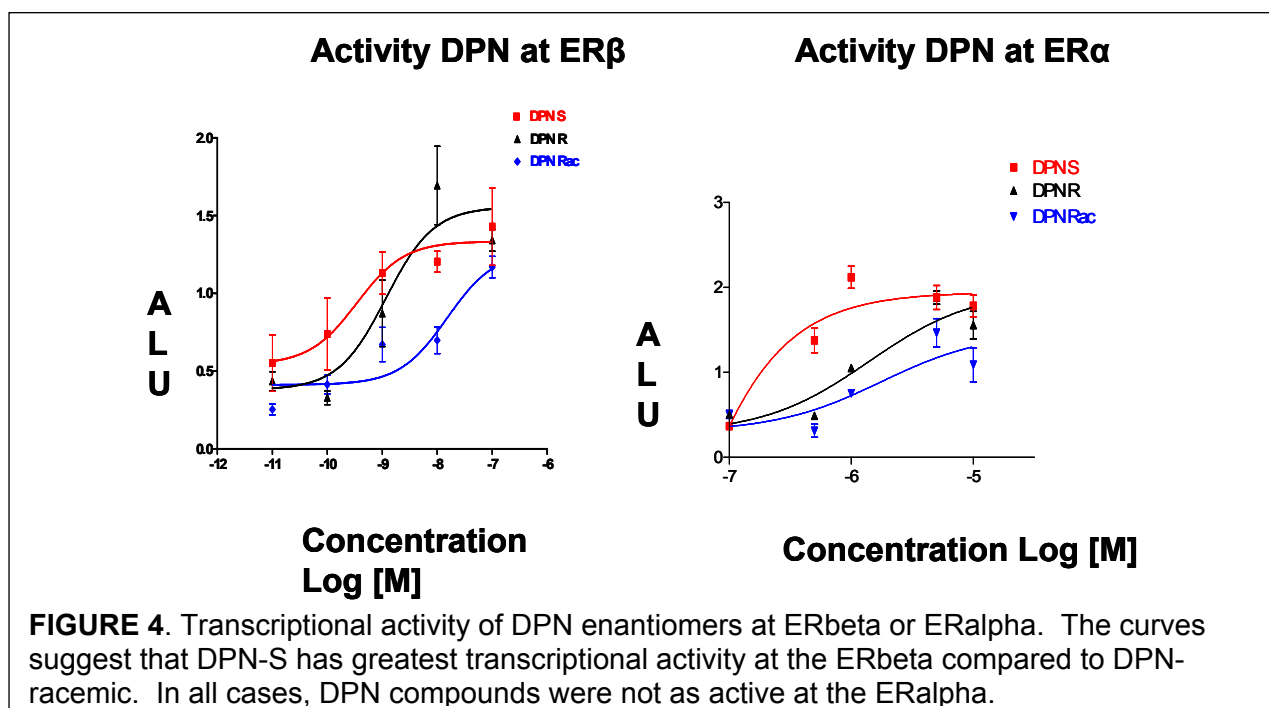
Approach:

It is essential that transcriptional efficacy also be determined for each of these compounds since binding affinity does not necessarily correlate with transcriptional efficacy. Furthermore, binding affinity does not indicate whether an individual compound possesses agonist or antagonist activity. To examine transcriptional efficacy, we used standard luciferase reporter gene assays. The human endometrial adenocarcinoma cell line (HEC-1-A) (ATCC, Manassas, VA) was used for all transient transfections. Cells were plated at a density of 0.4×10^5 cells/well in 24-well plates for 48 hours prior to transfection. Transfections were conducted using FuGENE (Roche Applied Science, Indianapolis, IN).

Cells were incubated with transfection media for 2 hours, followed by overnight recovery in serum-containing media. The next day, cells were incubated for 6h in serum-stripped media. Cells were then treated with increasing concentrations of DPN-racemic, DPN-S or DPN-R for 24 hrs and then cells were lysed. Luciferase activity was measured using 20 μ l lysate added to 100 μ l luciferin substrate (Promega Corp., Madison, WI). B-galactosidase activity was measured using 40 μ l lysate added to 200 μ l galacton substrate (Tropix-GalactoLight kit, Applied Biosystems). Luciferase activity was measured using a 20/20 TD luminometer (Turner Designs). Data are expressed as Arbitrary Light Units (ALU) calculated as a fold difference compared to 17 β estradiol (10 nM).

Results:

These data demonstrate that both DPN-S and DPN-R are agonists and both are more effective than DPN-racemic at inducing transcription of a reporter gene through an ERE. None of the compounds are very active through ERalpha and require doses of greater than 500 nM for activity. In all cases, the DPN compounds were approximately 500-1000 fold selective for ERbeta.



The second generation ERbeta agonist, DPN-S, alleviates anxiety.

Approach:

To test the effect of the second generation ERbeta agonist on anxiety, animals were tested in the elevated plus maze after four daily injections of test substances. The elevated plus maze is selected for these screening studies because it is rapid (5 min) and does not require any prior or extensive learning trials. Additionally, the elevated plus maze has strong claims to validity as testing an animal model of anxiety, both in a theoretical basis and as a drug response (Handley & McBlane, 1993). The model has an advantage in detecting anxiolytic and anxiogenic agents under the same operating conditions. The test measures a range of behaviors induced by anxiety and exploratory processes and thus it is very suitable to detect a broad variety of (even subtle) effects (Crawley & Palor, 1997).

Another way to gauge anxiety is to determine the animal's activity in an Open Field test (Figure 7) (Handa et al., 1997). In this assay, the rats were placed in a novel environment (100 cm x 100 cm x 20 cm) with a 40 W bulb at 100 cm above the test chamber. Within the test chamber is also a novel object. The rats were allowed to roam freely during the test and their behaviors are recorded by videotape. This was not originally proposed but we felt that it is an important experiment to conduct. In this study, individual animals were placed in an open field with a novel object in the center. More anxious animals spend less time in the center and spend less time exploring.

Results:

In the elevated plus maze, decreased anxiety is marked by the increased amount of time spent in the open arm and by the entry into the open arm. The rats treated with DPN-S and DPN spent more time in the open arms as well as had more entries into the open arms ($p < 0.05$) suggesting that DPN and DPN-S were effective in alleviating anxiety in the rats whereas PPT and DPN-R did not have an effect as we would have predicted (Figure 5).

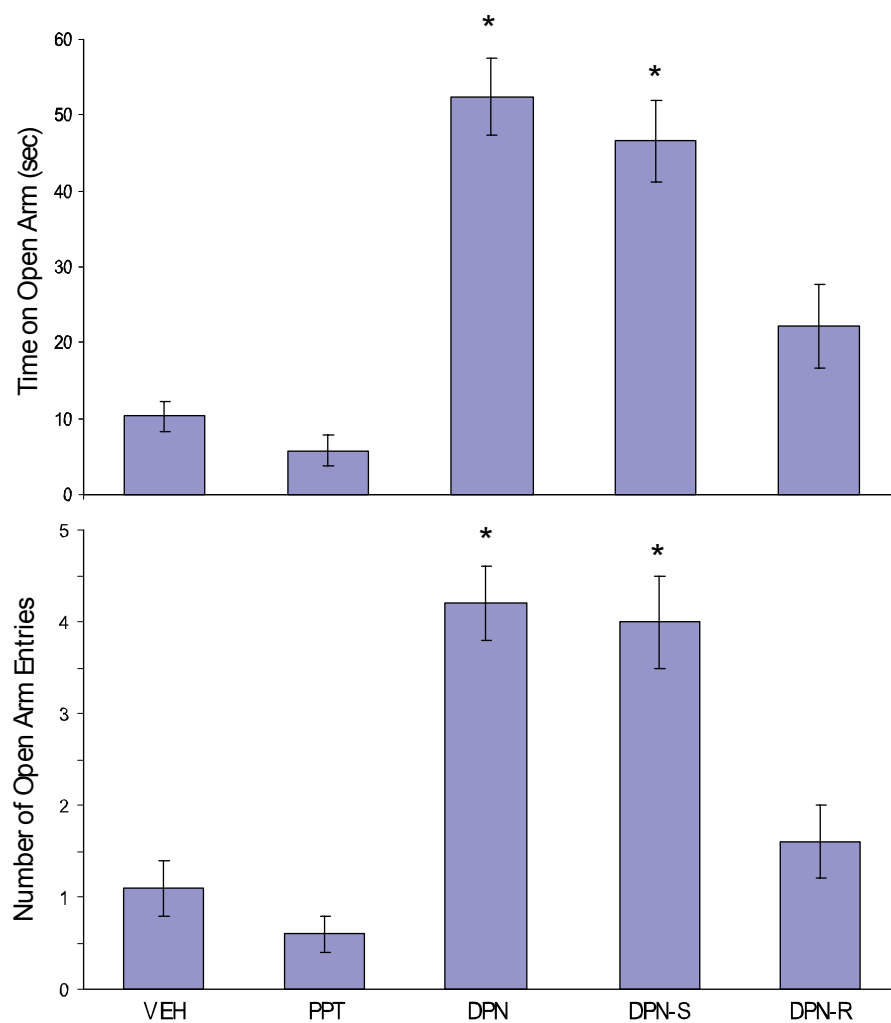


Figure 5. Animals treated with DPN and DPN-S spent greater time in the open arms (upper figure) and had greater numbers of open arms entries (lower figure) to suggest that both are good anxiolytics (*, $p < 0.05$ vs VEH).

We next determined the dose effect of DPN-S on anxiety using the elevated plus maze as the assay (same as Figure 5). Our data showed that rats treated with DPN-S and DPN-S- $\frac{1}{2}$ (for half dose; DPN and DPN-S were administered at 1 mg/kg bw/day, s.c. and half-dose was at 0.5 mg/kg bw/day, s.c.) spent significantly more time in the open arm of the elevated plus maze ($p < 0.05$) compared to those rats treated with VEH. In addition, the time spent for rats treated DPN, DPN-S and DPN-S- $\frac{1}{2}$ were not statistically different ($p > 0.10$) suggesting that they had equal efficacy.

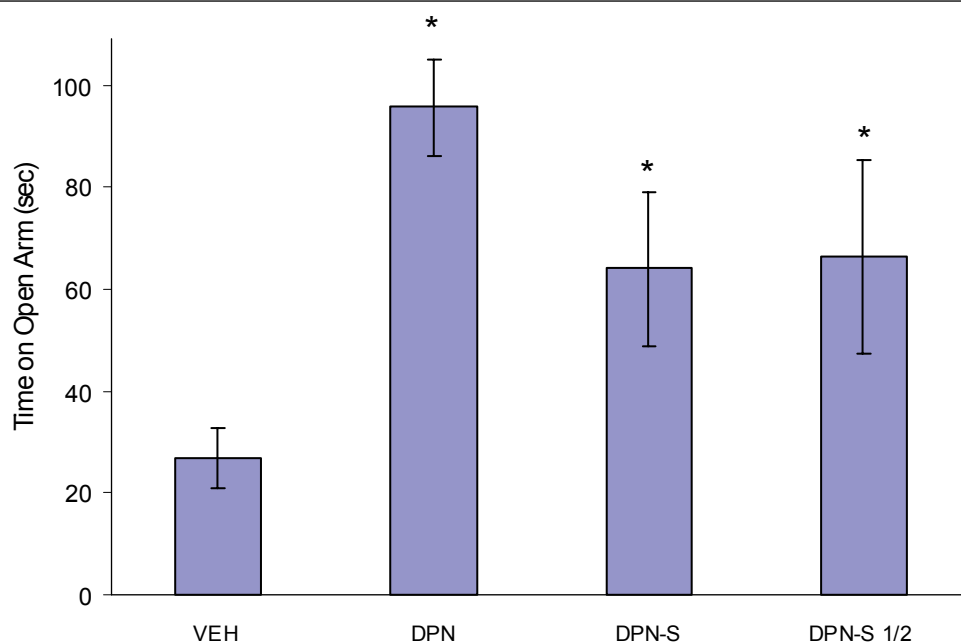


Figure 6. Effect of vehicle, DPN, DPN-S and DPN-S- $\frac{1}{2}$ on anxiety in rats. Animals treated with DPN, DPN-S and DPN-S- $\frac{1}{2}$ spent greater time in the open arms to suggest that DPN and DPN-S may be good anxiolytics (*, $p < 0.05$ vs VEH).

In this study, the rats treated with DPN or DPN-S spent more time ($p<0.05$) in the center of the open field compared to those treated with VEH and DPN-R. This suggests that the DPN and DPN-S treated rats are less anxious. In addition, rats given DPN and DPN-S also spent more time ($p<0.05$) exploring a novel object located randomly close to the center of the Open Field when compared to the VEH-treated rats.

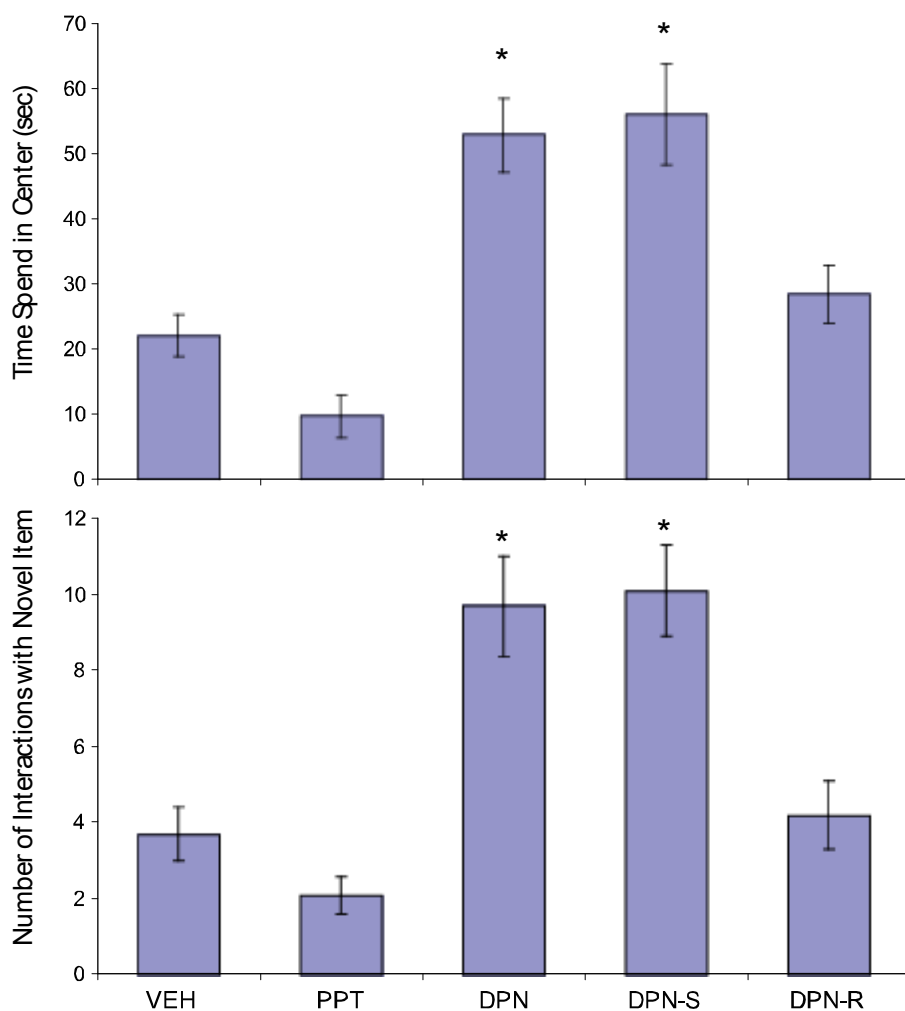


Figure 7. Effect of vehicle, PPT (an ER α agonist), DPN, DPN-S and DPN-R on open field behavior in rats. Animals treated with DPN and DPN-S spent greater time in the center of the open field (upper figure) and had greater numbers of interactions (lower figure) to suggest that DPN and DPN-S may be good anxiolytics (*, $p<0.05$ vs VEH).

Objective 1e. Effect of Second Generation ERbeta agonist, DPN: Cognition

Approach:

The Barnes maze is similar to the Morris Water Maze (MWM) for testing spatial learning in general, and reference memory vs. working memory in particular. However, the Barnes Maze produces less stress and physical demand on the animal and requires fewer trials for satisfactory training. A significant difference from the MWM is that the Barnes maze lacks the aversive component of increased adrenal activation and corticosterone release. The Barnes maze apparatus consists of a 48 inch diameter gray circle with 20 evenly spaced 2 inch diameter holes. The maze is placed upon a base that was 3 feet above the floor and was designed to use bright spotlights as the aversive stimuli. One of the holes has a target or “escape” box underneath it so the animal can escape from the brightly lit open platform. The key feature of the Barnes Maze was the inclusions of false boxes that are too small to entered but look the same as the target box. This system was used with a video tracking system to record the test data. Analysis of the data were done by ANY-maze version 4.2 software (Stoelting Company, Wood Dale, IL). Barnes Maze testing required that the position of the target box remained in a constant position relative to the room, although the platform itself were rotated to prevent confusion from any possible scent trails. Testing were conducted between 1 and 3 hrs after lights out. Animals were acclimated to the apparatus on the first day by being directly placed in the escape box and allowed to remain there for 2 minutes and then being provided an escape box. Testing began on the second day and consisted of three trials per day for additional 3 days. Animals were placed in a holding arena in the center of the platform for 30 seconds after its removal and the clock started and the experimenter moved behind the curtain. Testing was completed when the animal places all four paws into the escape box or when a pre-determined time (4 minutes) was reached. Each animal also spent 30 seconds in the escape box at the end of the testing session. There will be a 20 minute rest period between each trial. Feces were removed and the apparatus cleaned with an alcohol solution to remove odor cues.

Results:

In the Barnes maze, the results show that rats treated with DPN and DPN-S exhibited faster ($p<0.05$) times to locate the “escape” box when compared to the VEH-treated rats within 1 day of testing and continued to exhibit the same memory throughout testing (Figure 8). This is consistent with prior studies with estradiol (E2) showing that rats treated with estradiol have an enhanced memory. Interestingly, both DPN-S- $\frac{1}{2}$ and DPN-R but not DPN-R- $\frac{1}{2}$ also showed a tendency ($p<0.10$) to better recall the location of the “escape” box in the first day when compared to VEH-treated rats. One possible reason that the rats treated DPN-S- $\frac{1}{2}$ did not display better memory recall compared to VEH-treated rats on day 2 and day 3 of testing may be that that VEH-treated rats also improved over time.

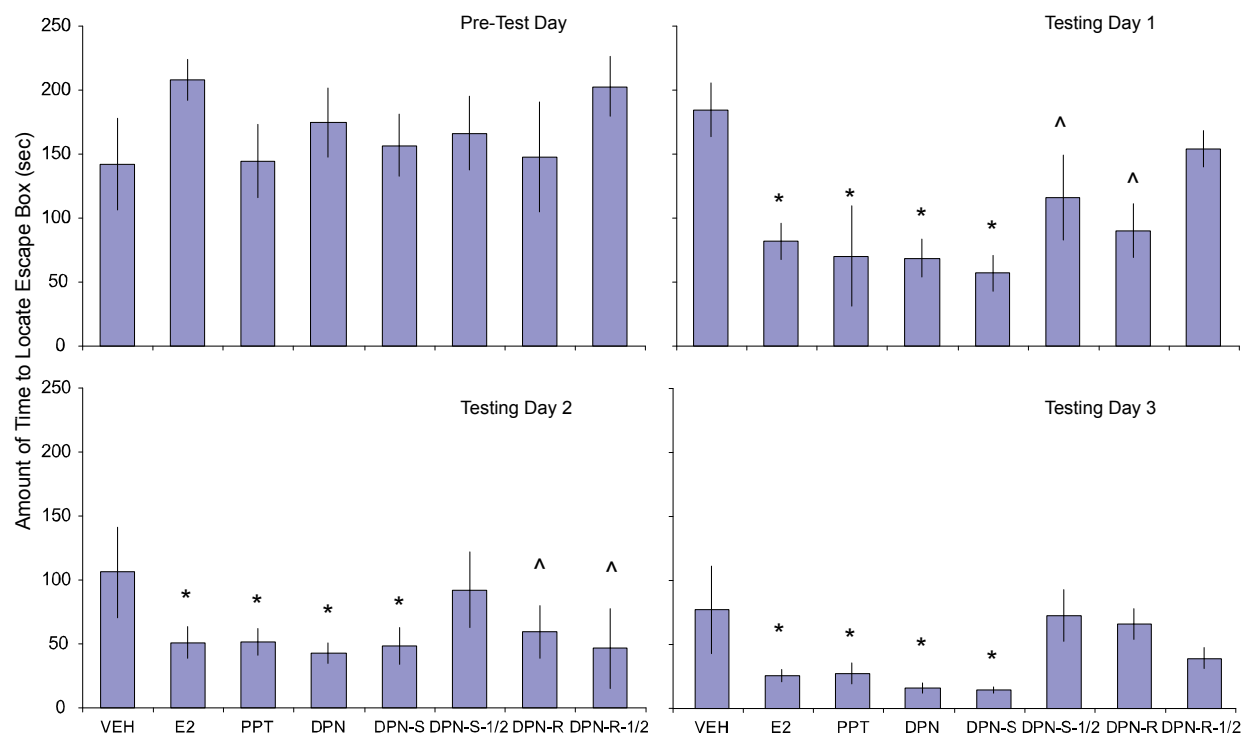


Figure 8. Effect of vehicle, estradiol (E2), PPT (an ERalpha agonist), DPN, DPN-S and DPN-R on memory in rats tested in the Barnes Maze. Animals treated with DPN and DPN-S spent less time locating the escape box on all 3 testing days. E2 and PPT-treated rats also showed the same memory recall. *, $p < 0.05$; ^, $p < 0.10$ vs VEH.

The second generation ERbeta agonist, DPN-S, improves depressive behavior

Approach:

The forced swim test is the classical test for depression and for the ability of drug-treatments to alleviate depression. In this test, rats were individually placed in a cylindrical tank measuring 60 cm height $\times$ 38 cm width. The tank was filled with water (24 ± 1 °C) at a height of 40 cm and the water was changed after each animal. The animals were forced to swim for a 15-min period (session 1) and 24 h later they were subjected to a 5-min swimming session (session 2) (Lucki, 1997). The FST behavior was scored manually (on line) and the total duration (in seconds) of immobility, swimming and climbing was registered from the summation of the time recorded with the use of a computerized program. Rats were considered to show immobility when they floated without struggling and only made movements necessary to keep their heads above the water. Swimming was recorded when they actively swam around in circles. Climbing was considered when the rats were climbing at the walls of the cylinder. Following each swimming session, the rats were removed from the tank, carefully dried in heated cages and then returned to their home cages.

Results:

In the forced swim test, the results show that rats treated with DPN and DPN-S exhibited greater struggle time and less immobilization when compared to the VEH-treated rats (Figure 9). Rats treated with PPT and DPN-R were not different ($p>0.10$) in their performance compared to the VEH-treated rats.

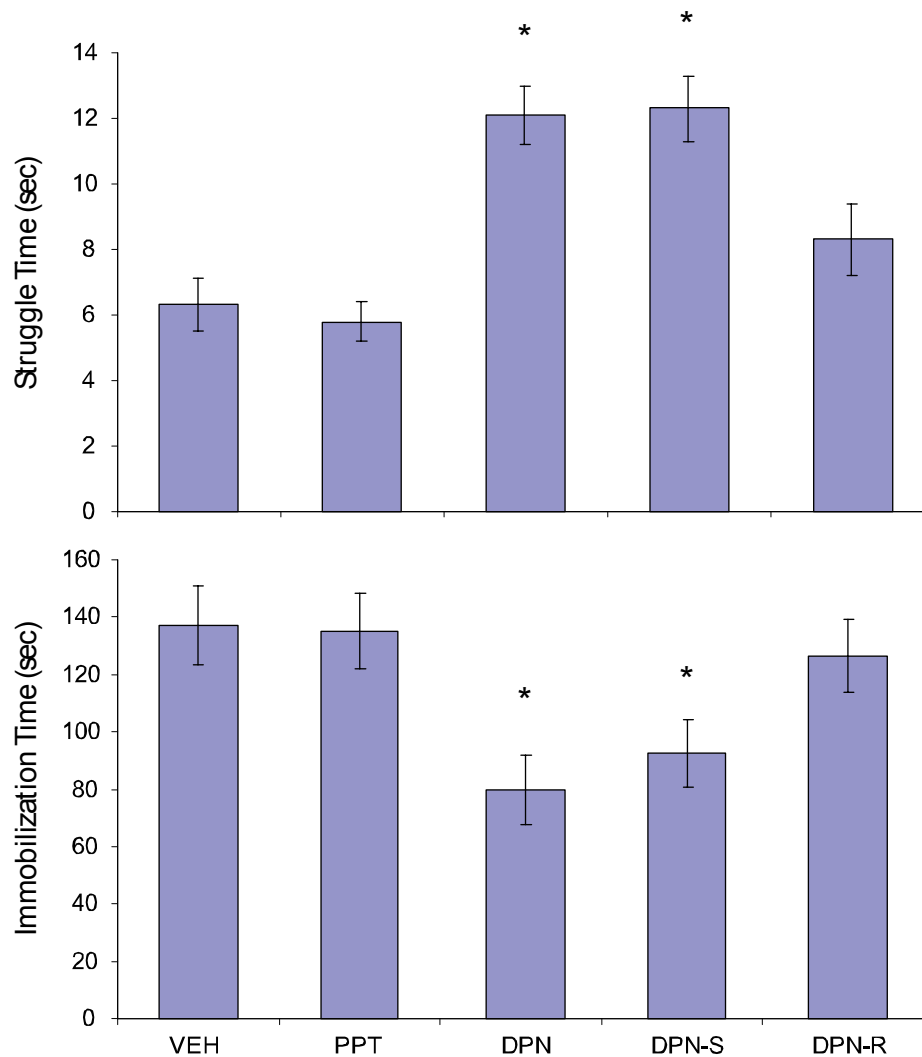


Figure 9. Effect of vehicle, PPT (an ER α agonist), DPN, DPN-S and DPN-R on struggle (top panel) and immobilization (lower panel) time in the forced swim test. Animals treated with DPN and DPN-S spent more time struggling and less time in immobilization compared to VEH treated rats. *, $p<0.05$ vs VEH.

Objective 2. To confirm that DPN and 2nd generation ERbeta agonists are devoid of side effects.

DPN and DPN-S does not have reproductive effects

Approach:

We approached this using two markers of feminizing effects of estradiol – 1. changes in body weight and 2. the ability to alter uterine size. It has been well described that the withdrawal of estradiol increases body weight and administration of estradiol decreases body weight. Conversely, estradiol can increase uterine weight (Ogawa et al., 1999; Wu et al., 2007). Both markers parallel reproductive abilities.

To determine if DPN had estrogenic side-effects, we examined the ability of 3 different doses of DPN-S, DPN-R or DPN-racemic to prevent weight gain that occurs following ovariectomy. Previous studies have demonstrated that post-ovariectomy weight gain is a consequence of the reduction in circulating estrogen titers. These results are shown in Figure 10. Animals were ovariectomized for 4 days and then injected daily (for 4 days) with estradiol benzoate (10ug/kg BW), or 3 doses of DPN-S, DPN-R or DPN-racemic (0.5, 1.0 or 2.0 mg/kg BW in vehicle). Body weight was monitored daily for the following 4 days and data are expressed as % change versus body weight immediately prior to the first injection.

At the end of the study, rats were killed and at necropsy, the uterus was dissected and weighted.

Results:

In our studies, DPN, DPN-S and DPN-R do not have an effect on body weight (Figure 10) and on uterine size (Figure 11).

In the present study, the rat's body weight increased following ovariectomy and this was prevented by treatment with estradiol benzoate (EB) ($p < 0.05$). DPN treatment of ovariectomized animals did not alter post-ovariectomy body weight gain ($p > 0.10$). Thus, these data indicate that neither DPN racemic, nor its S- or R-enantiomers possess the ability to alter body weight.

Converse to the effects on body weight, estradiol also has been shown to have an effect on reproductive function by regulating uterine weight. In this study, we determined that DPN, DPN-S and DPN-R did not have an effect on uterine weight ($p > 0.10$) ($p > 0.10$).

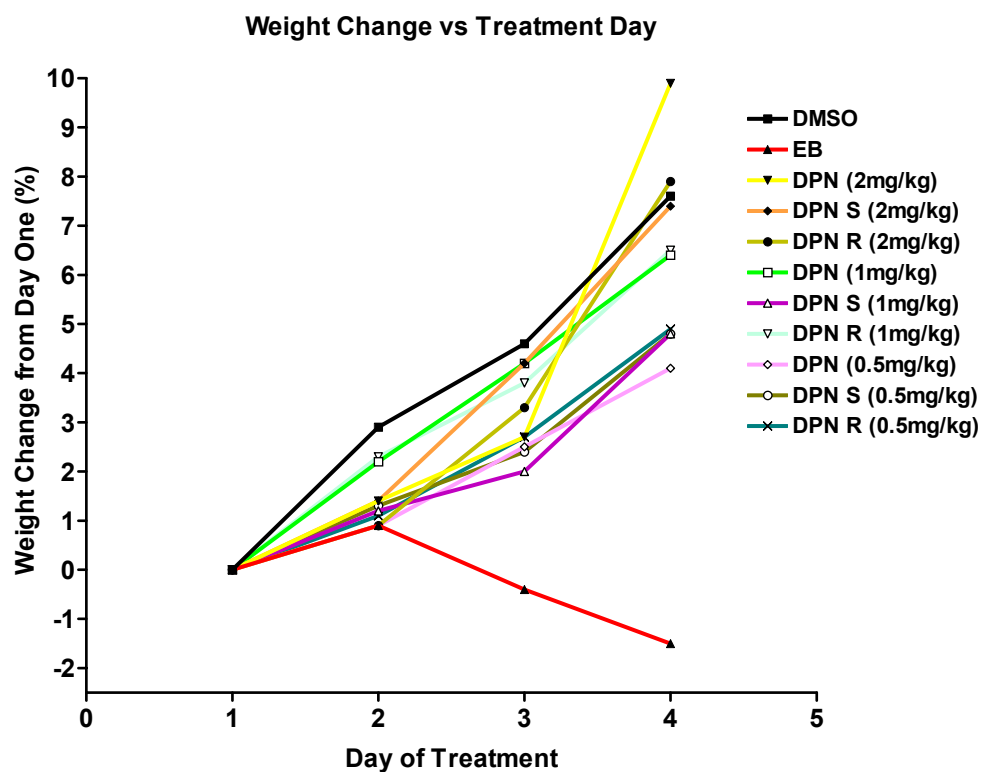


Figure 10. Post-ovariectomy body weight changes are prevented by estradiol, but not DPN treatment.

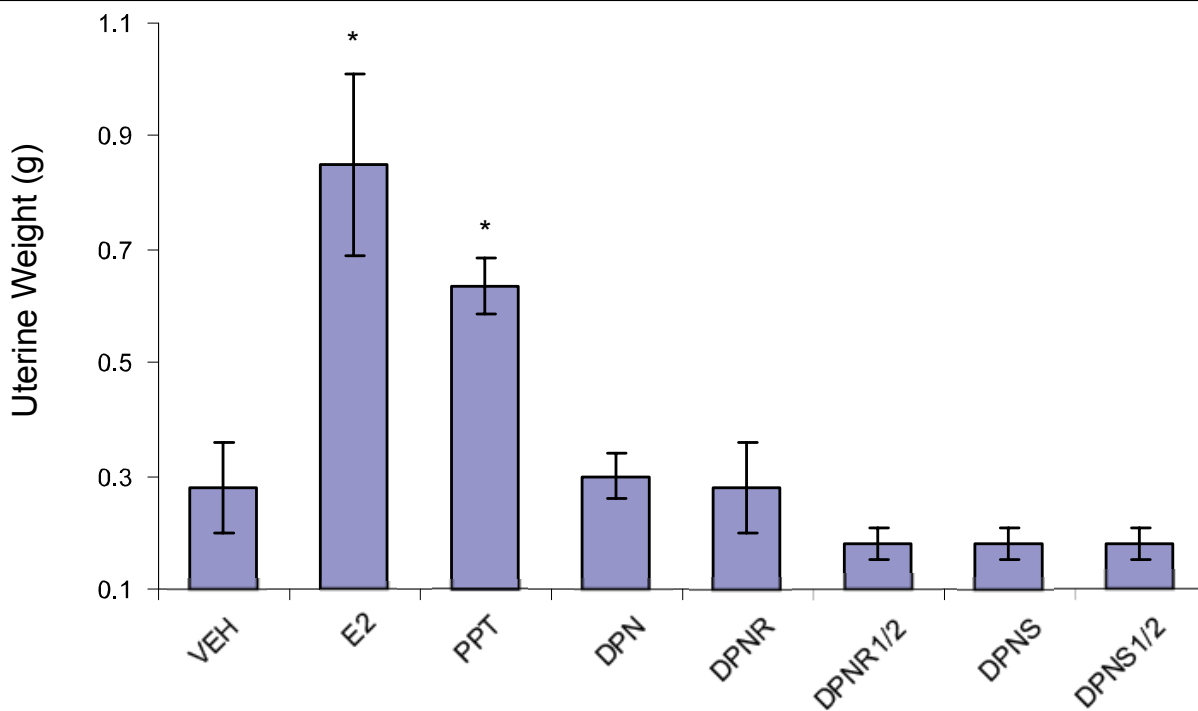


Figure 11. Post-ovariectomy uterine weight subsequent to treatment with VEH, E2, PPT, DPN, DPN-R, DPN-R-1/2, DPN-S and DPN-S-1/2. *, $p < 0.05$ vs VEH.

DPN and DPN-S does not have toxicological effects

Approach:

Carcasses obtained following sacrifice of animals were subjected to necropsy and histopathology. Histopathology will be performed on sections taken from liver and enzyme levels of LDH were determined in plasma to assess possible toxic effects of drug treatment.

Results:

Liver histopathology (data not shown) and plasma LDH levels (Figure 12) did not show any differences ($p>0.10$) between the groups.

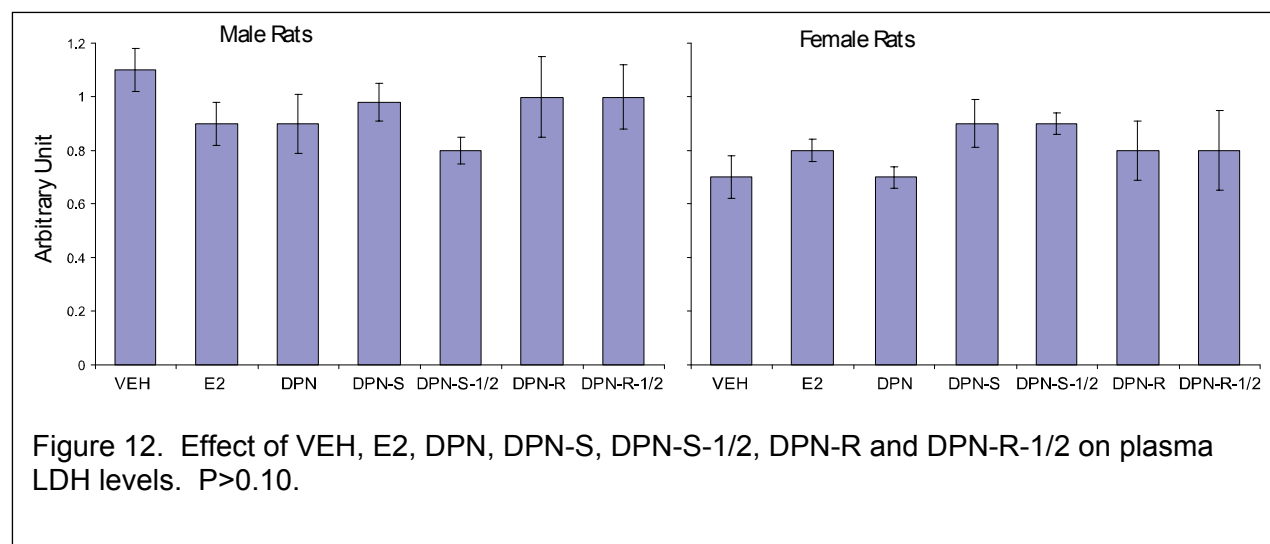


Figure 12. Effect of VEH, E2, DPN, DPN-S, DPN-S-1/2, DPN-R and DPN-R-1/2 on plasma LDH levels. $P>0.10$.

KEY RESEARCH ACCOMPLISHMENTS

Research Accomplishments:

Objective 1. To develop and test 2nd generation agonists with increased selectivity and affinity for ER β .

- a. We developed a second generation ERbeta agonist, DPN-S.
- b. We demonstrated that DPN-S has a higher binding affinity to ERbeta than DPN.
- c. We demonstrated that DPN-S has a greater transcriptional efficacy than DPN.
- d. We demonstrated that DPN-S can alleviate anxiety-related behavior.
- e. We demonstrated that DPN-S can improve cognitive function.
- f. We demonstrated that DPN-S can improve depressive behavior.

Objective 2. To confirm that DPN and 2nd generation ER β agonists are devoid of toxicologic side effects.

- a. We demonstrated that DPN and DPN-S does not affect body weight or uterine weight.
- b. We demonstrated that DPN and DPN-S does not appear to have toxicological side-effects as demonstrated by liver histopathology and plasma LDH levels.

c. REPORTABLE OUTCOMES

1. Abstracts

a. Society for Neuroscience 2007

Poster#: 730.20/QQ2

Title: Estrogen receptor beta activation leads to increased anxiolytic behaviors and decreased helplessness in female rats

Presentation Date: Tuesday, November 06, 2007

Authors: M. J. WEISER, T. J. WU, M. DAY, R. J. HANDA

b. Endocrine Society 2008 (abstract to be submitted January 15, 2007)

Title: Endocrine effects of SERMS in rats.

Authors: O.D. Larco, D.M Cruthirds, M.J. Weiser, R.J. Handa, T.J. Wu

c. Endocrine Society 2008 (abstract to be submitted January 15, 2007)

Title: Effect of diet on anxiety and body weight.

Authors: O.D. Larco, D.M. Cruthirds, M.J. WEISER, R.J. HANDA, T.J. Wu

d. Endocrine Society 2008 (abstract to be submitted January 15, 2007)

Title: Estrogen receptor activation leads to improved cognitive function in male and female rats

Authors: T.J. WU, M.J. WEISER, R.J. HANDA

2. Manuscripts

a. Enantiomer-specific effects of estrogen receptor beta receptor agonist diarylpropionitrile on anxiety and learned helplessness in the female rat (manuscript in preparation)

b. Estrogen receptor activation leads to improved cognitive function in male and female rats (manuscript in preparation)

c. Wegorzewska, I.N., K. Walters, M.J. Weiser, D.F. Cruthirds, E. Ewell, D.O. Larco, R.J. Handa and T.J. Wu (2008) Postovariectomy weight gain in female rats is reversed by estrogen receptor a agonist, propylpyrazoletriol. American Journal of Obstetrics and Gynecology (in press)

d. T.J. Wu, O.D. Larco, D.M. Cruthirds, M.J. WEISER, R.J. HANDA Effect of diet on anxiety and body weight. Experimental Biology and Medicine (manuscript in preparation)

3. Employment

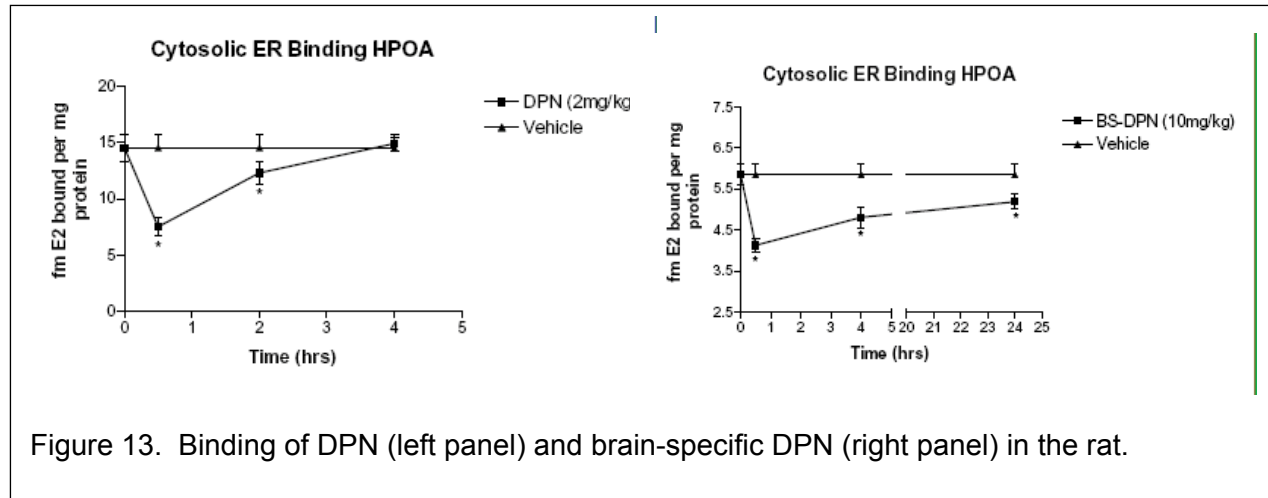
The PI of the proposal was promoted to Associate Professor with Tenure.

CONCLUSIONS

Summarize the results to include the importance and/or implications of the completed research and when necessary, recommend changes on future work to better address the problem. A "so what section" which evaluates the knowledge as a scientific or medical product shall also be included in the conclusion of the report.

The results of the present study suggest that pharmacological intervention via the ERbeta pathway may be a useful approach toward improving anxiety and cognition. The present studies further suggest that better agonists of ERbeta are warranted and may be used to enhance performance as well as to serve as a preventive tool when an individual is under extreme stress. The results, not only suggest that the conceptual approach of targeting the ERbeta pathway is important, but also suggest that a broader approach to designing a brain-specific agonist may be critical for clinical therapeutic.

We have some preliminary results to this extend. Below is data from a preliminary study showing that a single injection of a brain-specific DPN is longer acting (able to displace estradiol in the brain for over 20 hours) when compared to regular DPN (binding activity in brain up to 4 hours). From a pharmacological point of view, this would suggest that single injections can replace multiple daily injections of the same compound (Figure 13).



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List all references pertinent to the report using a standard journal format (i.e. format used in Science, Military Medicine, etc.).

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APPENDICES

1. Abstract presented at the Society for Neuroscience.
2. PI Curriculum Vitae.
3. Manuscripts and future abstracts to be submitted.

Program#/Poster#: 730.20/QQ2
Title: Estrogen receptor beta activation leads to increased anxiolytic behaviors and decreased helplessness in female rats
Location: San Diego Convention Center: Halls B-H
Presentation Start/End Time: Tuesday, Nov 06, 2007, 4:00 PM - 5:00 PM
Authors: *M. J. WEISER¹, T. J. WU², M. DAY³, R. J. HANDA¹;
¹Biomed Sci., Colorado State Univ., Fort Collins, CO;
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Estrogen has been shown to have positive and negative effects on anxiety and depressive-like behaviors. This dichotomous action of estrogen could be explained by the existence of two distinct estrogen receptor (ER) systems, ER alpha (ER α) and ER beta (ER β). In brain, ER α plays a critical role in estrogen regulating reproductive neuroendocrine function and behavior, whereas recent studies have suggested a role for ER β in anxiety and mood. To determine whether estrogens' anxiolytic and anti-depressant effects are via ER β , we examined the effect of several ER β -selective ligands on anxiety-like behaviors using the elevated plus maze (EPM) and open field (OF) test, or helplessness, using the forced swim test (FST). Young adult female Sprague-Dawley rats were ovariectomized and one week later administered one of several selective ER β agonists: racemic diarylpropionitrile (racemic DPN), the S enantiomer of DPN (S-DPN), the R enantiomer of DPN (R-DPN), WAY-200070 (Wyeth, Princeton, NJ), or the ER α agonist propylpyrazoletriol (PPT), or vehicle daily for seven days. In vitro binding studies utilizing recombinant ER β revealed that S-DPN has a 6.7 fold greater relative binding affinity (RBA) for ER β than does R-DPN. After four and five days of treatment, anxiety-type behaviors were measured on the OF and EPM, respectively. After seven days of treatment, depressive-like behavior was examined during the FST. Rats treated with racemic DPN, S-DPN, and WAY-200070 showed significantly decreased anxiety-like behaviors in both the open field and elevated plus maze. In the OF, these animals made more rears, interacted more with a novel object, and spent more time in the middle squares of the OF arena than did control, PPT, or R-DPN treated animals ($p < 0.01$). In the EPM, racemic DPN, S-DPN, and WAY-200070 treated females had significantly higher open arm entries, open arm time, rearing and head dips than did control, PPT, or R-DPN treated animals ($p < 0.01$). Rats treated with racemic DPN, S-DPN, and WAY-200070 showed significantly less depressive-like behaviors in the FST. These animals spent significantly more time struggling, and less time immobile than did control, PPT, or R-DPN treated animals ($p < 0.01$). In concordance with the calculated RBA, these results demonstrate that S-DPN is the behaviorally active enantiomer of DPN. These studies also indicate that estrogen's positive effects on mood, including its anxiolytic and anti-depressive actions, are likely due to its actions at ER β and raise the possibility that selective ER β agonists can be used in the treatment of anxiety or mood disorders. Disclosures: M.J. Weiser , None; T.J. Wu, None; M. Day, None; R.J. Handa, None.

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USAMRMC 04182001

Peer-Reviewed Publications (senior author publications are marked by an asterisk)

- 1 Walters K., I.N. Wegorzewska, Y.P. Chin, M.G. Parikh, **T.J. Wu*** (2008) Luteinizing Hormone-Releasing Hormone I (LHRH-I) and Its Metabolite in Peripheral Tissues. *Experimental Biology and Medicine* (in press).
2. Wegorzewska, I.N. (co-first author), K. Walters (co-first author), M.J. Weiser, D.F. Cruthirds, E. Ewell, B.S., D.O. Larco, B.S., R.J. Handa, **T.J. Wu*** Hormonal and Body Weight Regulation by Specific Estrogen Receptor Modulators (SERMS) in the Ovariectomized Rat. *American Journal of Obstetrics and Gynecology* (in press).
- 2 Roberts, J.L., M.J. Glucksman, S.K. Mani, **T.J. Wu*** (2007) Luteinizing hormone-releasing hormone metabolism (LHRH) and its processed metabolite, LHRH-(1-5). *Trends in Endocrinology and Metabolism* 18:386-392.
- 3 Newmark, J., M. Voekel, B. Brandon, **T.J. Wu** (2007) Delayed Onset of Malignant Hyperthermia without Creatine Kinase Elevation in a Geriatric, *RYR1* Compound Heterozygous Patient. *Anesthesiology* 107:350-353.
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- 5 Walters, K., Y.P. Chin and **T.J. Wu*** (2007) A Processed Metabolite of Luteinizing Hormone Releasing Hormone has Proliferative Effects in Endometrial Cells. *American Journal of Obstetrics and Gynecology* 196(1):33.e1-5.
- 6 Baldwin, E.L., I.N. Wegorzewska, M. Flora, **T.J. Wu*** (2007) Regulation of Type II Luteinizing hormone-releasing hormone (LHRH-II) gene expression by the processed peptide of LHRH-I, LHRH-(1-5), in endometrial cells. *Experimental Biology and Medicine* 232:146-154.
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- 9 Eddington, D.O., E.L. Baldwin, J.H. Segars, **T.J. Wu*** (2006) Steroid hormone

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11. Shippey-S.H., III, C.M. Zahn, M.M. Cisar, **T.J. Wu**, A.J. Satin (2005) Use of the Placental Perfusion Model to Demonstrate Transplacental Infection in Congenital Chagas' Disease. *American Journal of Obstetrics and Gynecology* 192(2):586-91.
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Submitted/To be Submitted

- 1 T.J. Wu, O.D. Larco, D.M. Cruthirds, M.J. WEISER, R.J. HANDA Effect of diet on anxiety and body weight. *Experimental Biology and Medicine* (manuscript in preparation)
- 2 Cummings, D.M., J.A. Wu, D.O. Eddington, **T.J. Wu*** Immunocytochemical localization of Brx in the mouse brain. *Brain Research* (In preparation)
- 3 Woller, M.J., M.G. Williams, L. Schmidt, **T.J. Wu*** Regulation of pulsatile LHRH secretion from hemi-hypothalamic explants by the LHRH processed metabolite, LHRH(1-5). (In preparation)
- 4 Wegorszewska, I.N., O. Larco, E. Ewell, **T.J. Wu*** Neuroprotective effects of specific estrogen receptor modulators on paraoxon toxicity. (In preparation)
- 5 Enantiomer-specific effects of estrogen receptor beta receptor agonist diarylpriopionitrile on anxiety and learned helplessness in the female rat

Abstracts Presented

Oral platform and poster presentations (several in the last 6 years) at the Endocrine Society, Society for Neuroscience and American Society for Reproductive Medicine.