The Effects of Environmental Chemicals on Neuroendocrine Function: Mode and Mechanism for Adverse Outcomes

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Contributing Organizations: NHEERL, NCCT, NCSU

LTG 1 Poster 05

Science Questions

Can a strategy be developed that will provide the means to determine the neuronal/molecular targets for environmental toxicants that alter the hypothalamic/pituitary control of gonadal function?

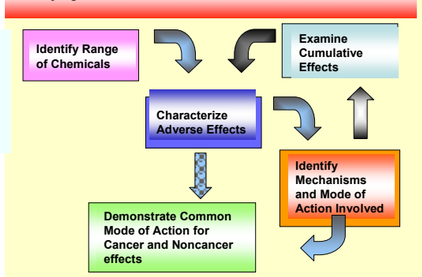
Research Goals

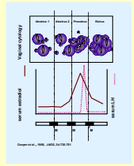
The hypothalamus and pituitary play critical roles in reproduction. In the female rat (and human), the mid-cycle (or ovulatory) surge of luteinizing hormone (LH) is a functional endocrine event that stimulates the final stages of follicular and oocytic maturation prior to ovulation. We have shown that environmental compounds from a variety of chemical classes are able to block this highly coordinated endocrine event. For example, the formamidine and dithiocarbamate pesticides block the catecholaminergic control of gonadotropin-releasing hormone (GnRH), and the chlorotriazine herbicides and intermediate metabolites affect tthe release of GnRH by a mechanism yet to be determined. We have also demonstrated that the disruption of the LH surge is a key event in the mode of action that leads to adverse reproductive outcomes including impaired pregnancy maintenance, impaired embryo development, reduced fertility, delayed puberty, premature reproductive senescence and mammary gland tumors in the female.

Due to the complexity of the observed toxicant-induced disruption of the neuroendocrine control of LH secretion and the numerous mechanisms that can be involved, we are developing a strategy to rapidly identify those cellular and molecular targets within the brain-pituitary that are affected by exposure to a test chemical. This strategy combines a series of in vitro (cell culture) and ex vivo (tissue perifusion, brain slices) procedures to identify the numerous mechanisms associated with the disruption of pituitary-gonadal and pituitary-adrenal function. We are coordinating our test chemical selection in order to integrate our results with those of the National Computational Center for Toxicology's ToxCast Research Program based on the NovaScreen® database). This approach will provide the information needed to determine the mode of action underlying the adverse effects of the chemical and provide the basis for across species comparisons. A related effort examines the extent to which toxicant-induced changes in the pituitary-adrenal axis contribute to alterations in reproductive physiology.

Identifying the Range of Chemicals

Effects of Environmental Agents on Neuroendocrine Function: Identifying Common Modes of Action and Adverse Outcomes





A number of xenobiotics disrupt the regulation of luteinizing hormone (LH).

- What are the causes [target organ(s), molecular sites]?
- What are the consequences (reproductive and non-reproductive outcomes)?

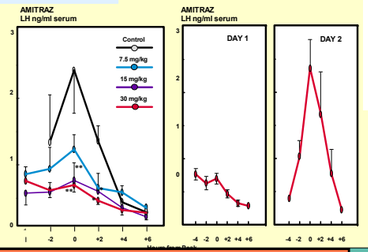
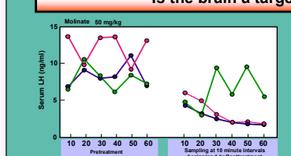


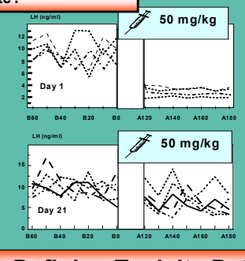
Table 1. Range of Chemicals That Modify the LH Surge

Formamidines	**Chlorotriazines**
Chlordimeform	Atrazine (+)
Amitraz	Propazine (+)
Diphenoxy Herbicides	Diaminochlorotriazine (+)
2,4-D (diphenoxy- (-) aceticacid)	Desethylatrazine (+)
Thiocarbamates	Deisopropylatrazine (+)
Molinate	Simazine (+)
Triallate	Ametryn (-)
Dithiocarbamates	**Organophosphates**
Thiram (+)	Malathion
Metam Sodium	**Haloacetic Acids**
Ferbam	Dibromoacetic Acid
Sodium-Dimethyldi-thiocarbamate	**Pharmaceuticals**
Organophosphate	Sodium Valproate
Malathion	Triclosan (-)
	Pyrethroids
	Permethrin
	Deltamethrin
	Cypermethrin
	Fenvalerate

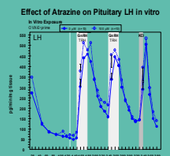
Red letters indicate positive effect on LH surge.
(+) effect or (-) no effect on onset of puberty.

Is the brain a target site?



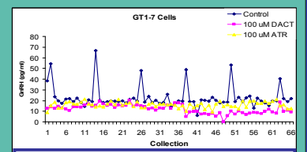


Effects of molinate on the pulsatile releae of LH in 3 ovariectomized rats. ! Attenuation of the pulses indicates the CNS mechanisms regulating gonadotropin-releasing hormone are changed.



Pituitary perifusion. Above, no differences in LH secretion between ex-vivo pituitaries of control or atrazine treated females. Below, there pituitary ACTH was not affected by atrazine when perifused *in vitro*

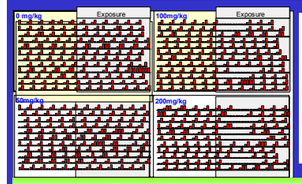
GnRH Neuronal Activity after Chlorotriazine Exposure



Effect atrazine or diaminochloro-triazine (100uM/24h) on GnRH pulsatility in GT1-7 GnRH neuronal cells. ● Both chemicals inhibited the pulses indicating that GnRH neurons are sensitive to the chlorotrizines

Characterizing Adverse Outcomes

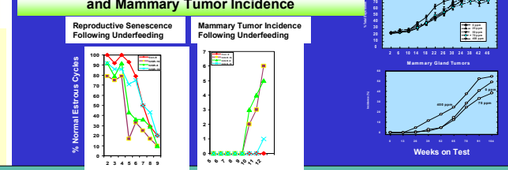
Evaluating Ovarian Cycles using Vaginal Cytology



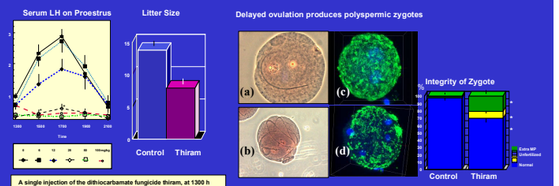
Metam Sodium: Daily gavage with this dithiocarbamate induced a dose-related increase in persistent vaginal diestrus indicative of impaired ovarian function.

Estrous cyclicity is a required endpoint in standard multigenerational protocols. Importantly, for metam sodium, but not all LH-disrupting chemicals tested, the system adapts to the insult, indicating that for certain compounds the extended exposures commonly employed in toxicological testing would not identify more proximal alterations in reproductive activity.

Correlation Between Reproductive Senescence and Mammary Tumor Incidence



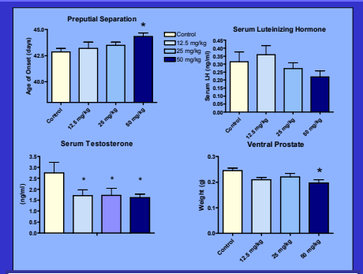
Evaluating Oocyte Function and Fertility



A single injection of the dithiocarbamate fungicide thiram, at 1300 h on vaginal proestrus decreases norepinephrine synthesis, suppresses the LH surge and delays ovulation for 24 h. This delay in ovulation caused a reduced litter size.

Polyspermic zygotes exhibit an abnormal (Type II & III) pattern of cortical granule exudate suggestive of a relationship between abnormal cortical reaction and polyspermy in the delayed zygote.

Evaluating Effects of Comp;ounds on Pubertal Develoment



Effects of thiram on pubertal development in male rats. ● Altered hypothalamic catecholamines interferes with the secretion of luteinizing hormone and results in a decrease in testosterone release. This decreased testosterone results in delayed PPS and decreased growth of androgen-dependent tissues.

For effects of other chemicals on pubertal development, see Table 1.

Impact and Outcomes

By demonstrating that the brain is a target site for several new chemicals, we unveiled a new mode of action for consideration in reproductive risk assessment.

By establishing parallel dose-relationships among effects on hypothalamic catecholamines, GnRH neuronal activation and the LH surge in response to DMDC, our work has identified the toxic pathway for the adverse effect of this class of metal-chelating pesticides on female reproductive function.

Our research strategy enabled us to elucidate common (or disparate) modes of action of atrazine metabolites and other chlorotriazine herbicides, information that contributed to the assessment of the cumulative risks of this widely used class of compounds by OPP and OW.

In vitro and *in vivo* studies defining the effects of atrazine and metabolites on aromatase and steroidogenesis have been key for interpreting recent reports in the literature suggesting that the adverse effects of this compound are mediated by alterations in aromatase activity in cell lines and wildlife species.

Furthermore, this research has now identified perturbations in the hypothalamic-pituitary-***adrenal*** axis on the reproductive tract as new area of concern.

Future Directions

- Further identify the extent to which changes in the adrenal axis mediate the adverse effects of xenobiotics on reproduction.
- Characterize the extent to which the HPA may mediate selected chemical-induced developmental dysfunctions (e.g., pubertal development, blood pressure changes etc).
- Characterize toxic effects on the relationships among the neuroendocrine factors contributing to the hypothalamic secretion of GnRH.
- Collaboration with NCCT investigators, using their databases, to more rapidly :

  identify the cellular mechanisms associated with altered neuroendocrine function, and

  identify compounds for further evaluation of their effects on these two important neuroendocrine systems

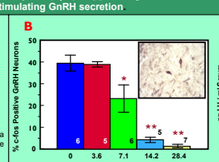
Defining Toxicity Pathways

Dithiocarbamates

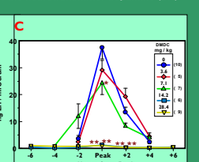
Compounds in this class of pesticides are metal chelators. In this case, sodium dimethyldithiocarbamate (DMDC) binds to the copper cofactor of the enzyme dopamine β-hydroxylase (DβH), suppressing the conversion of the neurotransmitter dopamine (DA) to norepinephrine (NE), which is involved in stimulating GnRH secretion

mg/kg DMDC	n	Posterior Hypothalamus NE	Posterior Hypothalamus DA	Anterior Hypothalamus NE	Anterior Hypothalamus DA	Caudate
0	5	13.5 ± 1.3	2.8 ± 0.4	18.0 ± 0.3	76.5 ± 5.0	
3.6	5	12.6 ± 0.4	3.0 ± 0.3	16.6 ± 1.5	82.5 ± 3.5	
7.1	5	10.6 ± 1.2	4.1 ± 0.3	15.1 ± 1.4	66.2 ± 6.4	
14.2	4	7.6 ± 0.8*	4.7 ± 0.2*	13.0 ± 1.6	87.9 ± 3.8	
28.4	5	6.2 ± 0.5*	6.2 ± 0.5*	8.3 ± 0.4*	88.3 ± 6.5	

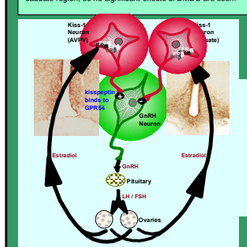
The dose-related fall in NE & rise in DA is indicative of a suppression in DβH. This enzyme is not present in the caudate region, so no significant effects of DMDC are seen.



Using an ovariectomized, steroid-primed animal model, the impact of DMDC on hypothalamic NE is paralleled by a suppression in the activity of GnRH neurons (using the presence of nuclear c-fos [black nucleus]- upper right) as an index of neuronal activation).

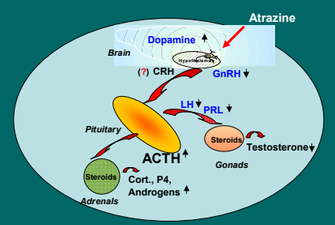


The effect of DMDC on the % of c-fos positive GnRH neurons is also paralleled by a suppression in the LH surge, defining the toxic pathway for this chemical.



The upregulatory effect of ovarian estradiol on the hypothalamus appears to be via binding to estrogen receptors (ERs) on Kiss-1 neurons (GnRH neurons do not contain ERs).Our ongoing research is exploring the effect of toxicants on the Kiss-1 secretory product kisspeptin, which in the anteroventral periventricular nucleus (AVPV), has been found to be the most powerful endogenous stimulus for GnRH release. In contrast, the binding of estradiol to arcuate Kiss-1 neurons has been reported to have a negative impact on GnRH secretion. This role in the hypothalamic regulation of pituitary gonadotropin secretion is still unclear.

Is the stimulation of the HPA-axis and inhibition of HPG axis due to a common mode of action?

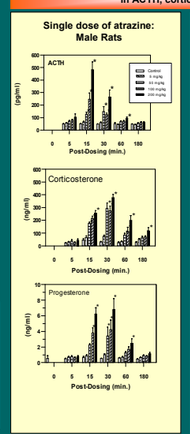


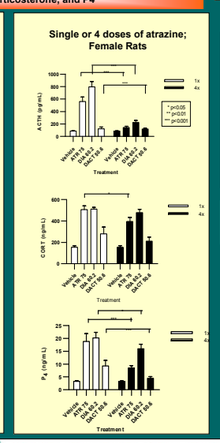
The observation that exposure to atrazine caused an elevation of corticosterone in male rats indicated that that this chlorotriazine herbicide was altering the hypothalamic-pituitary-adrenal (HPA) axis. This raised important questions that have been addressed:

- Are there dose-dependent changes in ACTH and Corticotropin Releasing Hormone (CRH)?
- Are the effects on the HPA mediated by a systemic or central MOA?
- Are the effects observed on the HPA and hypothalamic-pituitary-gonadal HPG) axis caused by a common mode of action?

Chlorotriazines

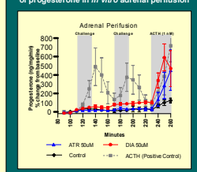
Atrazine stimulates dose and time-dependent increases in ACTH, corticosterone, and P4





Additional Evidence that Atrazine Alters Steroidogenesis

DIA, a metabolite of ATR, causes an elevation of progesterone *in vitro* adrenal perifusion

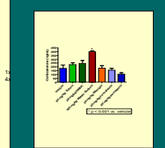


In vitro and *in vivo* studies support hypothesis that ATR and/or metabolites alter steroidogenesis.

Effect of Atrazine on Steroidogenesis

	H-295R Cell Culture	Granulosa Cell Culture	Male, *in vivo*
Progesterone	↑	↑	↑
Corticosterone	↑	NA	↑
Androstenedione	↑	NA	↑
Testosterone	=	NA	↑
Estradiol	↑	↑	↑
Estrone	↑	?	↑

Not all Chemicals that Alter the LH Surge Induce Adrenal Activation



Is there a common MOA for cancer and noncancer effects?

ATR as a Model Compound

- Increased CRH alters NE and GnRH release and inhibits LH release.
- Increased CRH stimulates ACTH and adrenal steroid production (including E, androgens) that may lead to premature reproductive aging/mamary tumors. (Pending outcome of adrenal androgen study in rats.)
- Increased CRH also increases CNS dopaminergic activity resulting in decreased prolactin secretion.
- Increased DA may cause increased free radical production and damage to DA neurons (Ramirez et al., 2005).

Other Researchers Involved

Michael Narotsky, John Laskey, David Dix, Keith Hauk, Angela Buckalew, Michelle Hotchkiss, Ashley Murr

Contributors: NHEERL: Goldman; Fraites; Narotsky; Laskey; NCSU Collect of Veterinary Medicine: Zorilla, Tinfo; NCCT: Dix, Hauk

Use of Mechanistic Data in Risk Assessment

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