ESTROGENIC ACTIVITY OF PERMETHRIN IN IMMATURE FEMALE RATS

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Introduction

Permethrin is a member of the synthetic type 1 pyrethroids, one of the newly developed classes of insecticdes due to its high activity against insects, and relatively low mammalian toxicity compared to other insecticide classes(1). Permethrin is widely used for in door pest control, providing potential for human exposure(2,3). *Garey et al* reported that permethrin showed no statisticaly significant antiestrogen activity in Ishigawa Var-1 human endometrial cancer cell line and the T47D human breast cancer cell line(4). It was reported that permethrin noticeably induced proliferation of MCF-7 human breast cancer cell (2). Also, *Saito K et al* demonstrated that permethrin showed no significant estrogenic or antiestrogenic effect *in vitro* using luciferase reporter gene assay and yeast two-hybrid assay(5). However, estrogenic or antiestrogenic potential of permethrin has been little known in both *in vivo* and *in vitro* as yet. Therefore, we examined the estrogenic potential of permethrin in immature Sprague-Dawley female rats using uterotrophic assay and level of Cabindin-D_{9K} (CaBP-9K) mRNA as the end point. Eighteen-day-old Sprague-Dawley female rats were subcutaneously treated with permethrin at dose levels of 10 to 800 mg/kg for 3 days. Treatment with permethrin resulted in increases in uterine weights at all doses tested and, doses of 200 and 800 mg/kg/day showed statistically significant increase in uterine weight. Similarly, treatment with permethrin induced the expression of Cabindin-D_{9K} mRNA at all doses tested, and this effect is statistically significant at dose of 800 mg/kg. Our results demonstrate that permethrin behaves like estrogen agonist in immature female rat and suggest that exposure to this compound may contribute to reproductive dysfunction or developmental impairment through this hormonal activity.

Methods and Materials

Animals. Eighteen day old Sprague-Dawley female rats were obtained from National Institute of Toxicological Research (NITR), and cared and processed in accordance with NITR guide for the care and use of laboratory animals.

Uterotrophic assay. Immature Sprague-Dawley female rats (PND18, about 61g) were randomly assigned to control and treatment groups 1 day before treatment. vehicle control (corn oil), positive control(17β-estradiol: E2, 3 ug/kg/day) or permethrin(10 to 800 mg/kg/day) were administered via subcutaneous injection for 3 consecutive days. Animals were sacrificed by cervical dislocation 24hr after the final treatment. The uteri were removed, trimmed free of fat and subsequently

weighed.

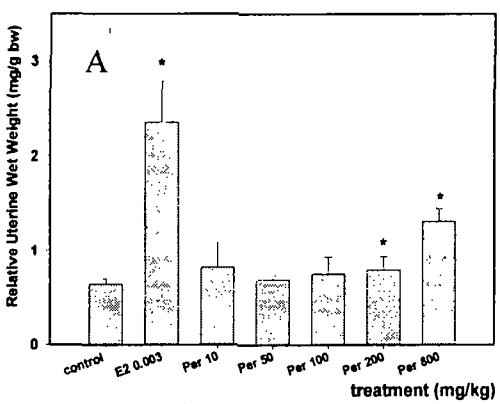
Dot blot analysis of Cabindin-D_{9K} mRNA expression. The uteri were obtained from uterotrophic assay and total RNA was extracted with Trizol (Life Technologies, inc.). 10μg of total RNA was electrophoresed on 1% agarose gels for 90 min at 110V. A dot blot assay was carried out at a loading concentration of 5μg. The membranes were hybridized with rat Calbindin-D_{9k} cDNA and exposed to X-ray films.

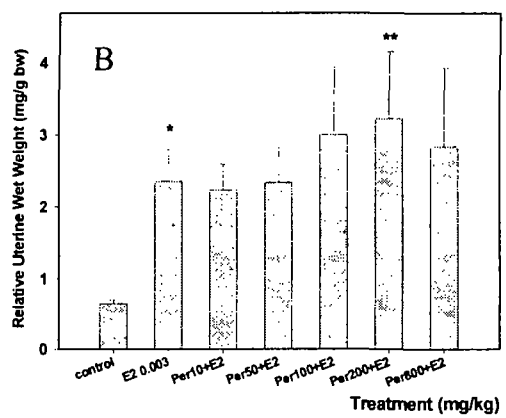
Statistics. The statistical analysis was performed by student's t-test. The significant differences between groups evaluated at level of 0.05.

Results and Discussion

Effect of permethrin on rat uterine weight. Figure 1 shows that effect of permethrin on rat uterine weight. The treatment with permethrin of 10, 50 and 100 mg/kg/day for 3 consecutive days resulted in statistically insignificant increases in rat uterine weight, whereas doses of 200 and 800 mg/kg/day brought significant increases. When permethrin was concomitantly treated with E2 enhanced the uterine weight stimulated by E2. These results suggest that permethrin behaves like estrogen agonist in immature female rats.

Effect of permethrin on expression of Calbindin-D_{9K} mRNA. Figure 2 shows that effect of permethrin on expression of Calbindin-D_{9k} mRNA. The treatment with permethrin of 200 and 800 mg/kg/day for 3 consecutive days led to significant induction of Calbindin-D_{9K} mRNA expression. When permethrin was concomitantly treated with E2, permethrin significantly stimulated the Cabindin-D_{9K} mRNA expression enhanced by E2. This effect was significant at E2 plus permethrin (200 mg/kg/day) group





Acknowledgements

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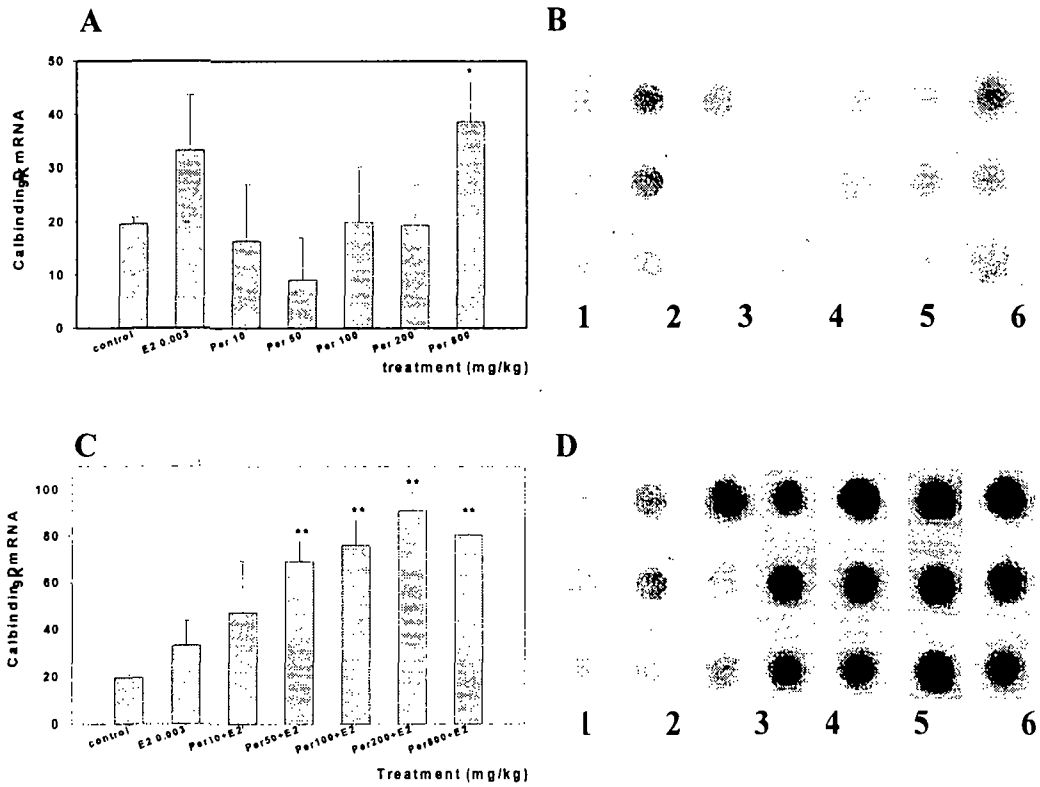
Figure 2. Induction of Calbindin-D_{9K} mRNA Expression in Immature Female Rats Treated with Permethrin or E2 plus Permethrin.



A: Schematic diagram of Calbindin-D_{9K} mRNA expression by permethrin. Each point represents mean±SD from 8 uteri. *Significantly different from control group at $P<0.05$.
B: Dot blot analysis of Calbindin-D_{9K} mRNA expression by permethrin. Lane 1; control, 2; E2, 3-6; permethrin 10, 50, 100, 200, 800, respectively.
C: Schematic diagram of Calbindin-D_{9K} mRNA expression by E2 plus permethrin. Each point represents mean±SD from 8 uteri. **Significantly different form E2 treatment group at $P<0.05$.
D: Dot blot analysis of Calbindin-D_{9K} mRNA expression by E2 plus permethrin. Lane 1; control, 2; E2, 3-6; E2 plus permethrin 10, 50, 100, 200, 800, respectively.