CHAPTER V

EFFECTS OF RESERPI NE AND α METHYL-p-TYROSINE (α-MPT) ON PLASMA GONADOTROPINS, PRL AND HYPOthalAMIC TH ACTIVITY IN OVX RATS

Reserpine is an alkaloid which interferes with the uptake of catecholamines into storage granules of axon terminals thereby depleting the brain of catecholamines (Kirschner, 1962; Von Euler and Lishajko, 1963). Most of the acute central nervous system (CNS) effects of this drug, such as catalepsy and parkinsonian signs are attributed to a loss of dopamine since they are readily reversed by L-Dopa (Blaschko and Chrusciel, 1939; Carlsson et al., 1957). The depletory effect is generally believed to result from impairment of the amine binding capacity of the storage organelle.
α-methyl-p-tyrosine specifically inhibits catecholamine biosynthesis at the tyrosine hydroxylation step, inhibition of that enzyme being competitive with the natural substrate tyrosine. As a result, dopamine as well as noradrenaline levels are lowered in both peripheral adrenergic tissues and in the CNS. Unlike that of reserpine, the depletory effects of α-MPT are singularly dependent on neuronal impulse activity (Corrodi and Malmfors, 1966). Most of its CNS depressant effects in animals such as depression of locomotor activity (Corrodi and Hanson, 1966) catalepsy (Bedard, 1970) disruption of conditioned behavior (Hanson, 1965) and amphetamine antagonism (Weissman et al., 1966) may be casually related to DA depletion, since they are readily reversed by L-Dopa. The effects of reserpine and α-methyl-p-tyrosine were evaluated by measuring changes in hypothalamic TH activity following the administration of reserpine or α-MPT and by correlating these changes with plasma gonadotropin and PRL levels.

Experimental procedure: Reserpine (Sigma Chemical Co., USA, Lot. 97C-0033) was dissolved in 20% ascorbic acid (10 mg/0.5 ml) and was administered sc in a dose of 10 mg/kg bw/day, for 3 days to a group of ovariectomized rats. Controls were given equal volume of vehicle. The animals were killed by decapitation, 24h after the last injection.
α-Methyl-p-tyrosine (Sigma Chemical Co., USA, Lot 582-0371) dissolved in 0.1 N HCl (200 mg/ml) (pH adjusted to 5.6–6.0 with 5 N NaOH) was administered in a dose of 400 mg/kg bwt, ip. Controls received equal volume of vehicle. Animals were decapitated 2h after injection. Plasma gonadotropin and PRL levels and hypothalamic TH activity were measured as described in Chapter II.

RESULTS

Gonadotropin, PRL and hypothalamic TH activity after Reserpine: Ascorbic acid diluent administration had no effect on plasma gonadotropin levels. Reserpine administration (10 mg/kg bwt/day, sc, for 3 days) produced only a slight decrease in plasma LH and FSH levels (Fig. 14). Plasma prolactin levels, however, were significantly (P<0.05) increased, following the administration of the drug for 3 days (10 mg/kg bwt/day, sc) when compared to the vehicle treated OVX rats (Fig. 15).

Hypothalamic TH activity was unaltered after the injection of the diluent. On the other hand, TH activity was significantly elevated (P<0.05) following reserpine injections (Fig. 15).

Gonadotropin, PRL and hypothalamic TH activity after α-MPT: Administration of α-methyl-p-tyrosine, an inhibitor of
Fig. 14. Plasma LH and FSH levels following the administration of reserpine (10 mg/kg bwt/day, sc, for 3 days) or α-methyl-p-tyrosine (400 mg/kg bwt, ip) in OVX rats.

*P<0.05 vs Control
Fig. 15. Plasma PRL levels and hypothalamic TH activity following the administration of reserpine (10 mg/kg bwt/day, sc, for 3 days) or α-methyl-p-tyrosine (400 mg/kg bwt, ip) in OVX rats.

* $P < 0.05$ vs Control
* * $P < 0.01$
* * * $P < 0.001$
tyrosine hydroxylase, (400 mg/kg bwt, ip) significantly suppressed (P<0.05) plasma LH levels at 2h after injection in OVX animals (Fig. 14). Plasma FSH levels were also significantly (P<0.05) suppressed following α-MPT injection with respect to the control values (Fig. 14).

α-Methyl-D-tyrosine injection (400 mg/kg bwt, ip) which specifically blocks tyrosine hydroxylase activity significantly elevated (P<0.01) plasma prolactin levels (Fig. 15).

Hypothalamic TH activity was significantly inhibited (P<0.001) following α-methyl-D-tyrosine treatment (400 mg/kg bwt, ip) with respect to values observed in control animals (Fig. 15).

**DISCUSSION**

Reserpine, a drug which blocks the uptake of catecholamines into storage vesicles, only slightly suppressed plasma gonadotropin levels, however, elevated PRL levels and increased hypothalamic TH activity following daily injections for 3 days. Reserpine is shown to block pre-ovulatory surge of LH (Barracough and Sawyer, 1957). This alkaloid, stimulates corticosterone synthesis (Van Loon, 1974) and inhibits thyroid function (Reichlin, 1966).
Earlier studies showed that administration of anti-
hypertensive drugs e.g. reserpine to patients resulted in
abnormally high levels of prolactin (Tolis et al., 1973).
The depletion of hypothalamic dopamine reserves by
reserpine apparently elevated PRL levels. Paradoxically,
hypothalamic TH activity was also increased by daily
injections of reserpine for 3 days. It has been noted
that reserpine depletes the brain of dopamine, norepine-
phrine and also serotonin (Shore et al., 1955).

Increased levels of tyrosine hydroxylase are observed
in homogenates of adrenal glands after 3 days of reserpine
administration (Thoenen et al., 1969; Mueller et al., 1969).
However, there is an abrupt reduction in the tyrosine
hydroxylase activity of intact mouse vas deferens prepara-
tion, following the acute administration of reserpine.
The effects of reserpine on catecholamine synthesis appear
to be complex and are determined by the tissue, the degree
of catecholamine depletion and duration of the reserpine
effect. Shortly after reserpine administration the acute
block of norepinephrine uptake into granules results in
enhanced end-product feedback inhibition of tyrosine hydro-
xylase and reduced activity of this enzyme. Prolonged
depletion of noradrenaline however, induces an increased
synthesis of tyrosine hydroxylase (Thoenen et al., 1969;
Mueller et al., 1969).
The enhanced hypothalamic TH activity may be attributed to autoregulatory feedback mechanism of PRL release, by which increased levels of prolactin stimulate the synthesis of TH. Cycloheximide, an inhibitor of protein synthesis, is reported to disrupt the PRL mediated stimulation of dopamine synthesis in these neurons (Johnston et al., 1980).

When pituitary glands are incubated in the presence of reserpine, there is a significant dose-related inhibition of PRL release, suggesting that pituitary action of this alkaloid is opposite to that of CNS action and this direct effect of reserpine to inhibit PRL release is reported to be independent of any interaction with catecholamine systems and is mediated by other, presently undefined mechanisms (Login and MacLeod, 1981).

α-Methyl-p-tyrosine, a specific inhibitor of the tyrosine hydroxylase, the rate limiting enzyme in catecholamine biosynthesis, inhibited the elevated gonadotropin levels in ovariectomized rats. Plasma prolactin levels, on the other hand, were significantly elevated. This substance has earlier been reported to block preovulatory discharge of LH and progesterone induced LH release in estradiol primed rats (Kalra and McCann, 1975).
The suppression of LH and FSH release following α-methyl-p-tyrosine may be due to the inhibition of noradrenaline synthesis in different areas of brain by this compound. Noradrenaline which strongly stimulates LH release, is implicated in the physiological control of LHRH-LH release (Krieg and Sawyer, 1976; Clifton and Sawyer, 1979). Prolactin levels were significantly elevated, apparently due to the inhibition of hypothalamic dopamine synthesis as evidenced by suppression of TH activity.