ROLE OF GAMMA AMINOBUTYRIC ACID (GABA) IN THE CONTROL OF
GONADOTROPIN AND PRL RELEASE IN OVX, CONSCIOUS RATS

The neutral amino acid GABA was initially identified
in brain by Awapara et al. (1950); Roberts and Frankel
(1950) and Udenfriend (1950). Four years later Hayashi
(1954) suggested that GABA might play a role in the regula-
tion of neuronal excitability and Florey (1954) discovered
that an extract of mammalian brain had an inhibitory action
on crayfish stretch receptor neurons. The active component
of the brain extract was identified as GABA by Bazemore
et al. (1957) and they suggested that GABA might be an
inhibitory transmitter in the mammalian nervous system.
Curtis et al. (1959) demonstrated that GABA produced hyper-
polarization and increased conductance. It is now well
accepted that GABA is a major inhibitory neurotransmitter
throughout the CNS (Dreifuss et al., 1969; Davidson, 1976) and modification of GABAergic inhibition has been determined to be involved in the actions of a number of drugs used clinically such as barbiturates (Nicoll, 1972; 1975) and benzodiazepines (Suria and Costa, 1975) in epilepsy (Meldrum, 1975) and Huntington's disease (Hornykiewicz, 1972).

Synthesis and degradation: Decarboxylation of glutamate produces GABA. Glutamate decarboxylase, the enzyme that catalyzes the formation of GABA is found in the tissues of central nervous system including hypothalamus and median eminence (Vincent et al., 1982). This enzyme requires pyridoxal phosphate as a coenzyme. GABA is further metabolized by deamination to succinic semialdehyde by the enzyme GABA-transaminase (GABA-T) which is also a pyridoxal dependent enzyme.

Potent drugs have been developed which increase or decrease GABA concentrations either by acting on metabolic pathways or by acting through the receptors. These include:

1) GABA - mimetic drugs:
   a) Muscimol: a potent GABA agonist with respect to bicuculline-sensitive post-synaptic receptors (Curtis et al., 1971).
   b) Guvacine: A substrate-competitive inhibitor of GABA uptake (Johnston et al., 1975).
c) Ethanolamine-o-sulfate: A specific catalytic inhibitor of GABA transaminase (Fowler and John, 1972).

d) Aminooxycetic acid (AOAA): Inhibitor of GABA transaminase.

2) Antagonists of GABAergic function: Picrotoxin and Bicuculline, which easily cross the blood brain barrier (Straughan et al., 1971).

High concentrations of GABA in the hypothalamus, median eminence and also within anterior posterior and intermediate lobes of pituitary have been demonstrated in mammals using autoradiographical and immunohistochemical methods (Fahn and Cote, 1968; Okada et al., 1971; Fahn, 1976; Vincent et al., 1982). Since hypothalamus is involved in the regulation of anterior pituitary hormone secretion, attempts have been made to indicate a role for GABA in the control of AP hormone release (Ondo, 1974; Vijayan and McCann, 1978). Consequently, we evaluated the effects of GABA on gonadotropin and prolactin release following its intraventricular administration in OVX, conscious rats.

Hypothalamic TH activity was also estimated following the administration of GABA, to evaluate the role of dopamine in the mediation of GABA action, if any.
Aminoxyacetic acid has been shown to increase brain GABA levels by inhibiting GABA-transaminase, the enzyme which catabolizes GABA to succinic semi-aldehyde (Collins, 1973; Wood and Peesker, 1973 and Perry et al., 1974). The effects of AOAA on plasma gonadotropin, and PRL levels and hypothalamic TH activity were also investigated.

**Experimental Procedure:** Rats were cannulated as described in Chapter II. Gamma aminobutyric acid (Sigma Chemical Co. USA, Lot 27C-0287) was prepared freshly in 0.9% NaCl and 0.1 or 4 μmol dose was microinjected into the 3rd ventricle in a volume of 2.5 μl. Controls received equal volume of saline. Five and 15 min after injection the animals were decapitated, brain was processed for TH assay and plasma from trunk blood was separated for the assay of gonadotropin and PRL by RIA (as described in Chapter II).

Aminoxyacetic acid (Sigma Chemical Co. USA, Lot 118C-0070) was prepared freshly in 0.9% NaCl (pH adjusted to 7.0) was injected ip 0.46 mmol/kg bw to OVX rats in a volume equivalent to 1% of the body wt. of the animal. Controls received an equal volume of saline. Animals were decapitated after 1h and hypothalamic TH and plasma gonadotropin and PRL levels were measured.

**Results:** Intraventricular Injection of GABA modified

**Initial plasma concentrations of gonadotropins and PRL:** Plasma gonadotropin (LH and FSH) titers were significantly
elevated and PRL levels were relatively low in OVX rats.

**Plasma gonadotropin levels following intraventricular GABA:**
Third ventricular administration of saline did not produce any changes in plasma hormone levels. Intraventricular injection of 0.1 μmol GABA had no effect on plasma LH levels at 5 or 15 min (Fig. 4). On the other hand, the higher dose of 4 μmol elevated LH levels (P<0.05) at 15 min. Third ventricular injection of either dose (0.1 or 4 μmol) of GABA failed to alter plasma FSH levels (Fig. 5).

**Plasma PRL levels:** Prolactin levels were lowered slightly at 5 min by 0.1 μmol dose of intraventricular GABA, whereas, the levels were significantly suppressed (P<0.05) at 15 min. On the contrary, the higher dose of 4 μmol produced a significant elevation (P<0.05) in plasma PRL concentrations (Fig. 6).

**Hypothalamic TH activity:** Hypothalamic TH activity was not modified by the intraventricular injection of saline. Third ventricular administration of either dose of GABA failed to induce any alteration in hypothalamic TH activity at 5 or 15 min (Fig. 7).

**Effect of Aminooxyacetic acid on plasma gonadotropin levels:** Intraperitoneal injection of 0.46 mmol AOAA/kg bwt, a dose which has been demonstrated to enhance
Fig. 4. Plasma LH levels after intraventricular injection of 0.1 or 4 μmol GABA in OVX, conscious rats at 5 and 15 min after injection. In this and subsequent figures, numbers at the base of each column or in parentheses indicate the number of animals in each group and/or at each point. Vertical lines above and/or below the mean represent mean ± SEM. Since there was no significant difference in hormone levels at 5 or 15 min following ivt injection of saline, these values were pooled.

*P<0.05 vs Control.
Fig. 5. Plasma FSH levels at 5 and 15 min after third ventricular administration of 0.1 or 4 µmol GABA.
Fig. 6. Plasma PRL levels at 5 and 15 min after 0.1 or 4 μmol intraventricular GABA.

*P<0.05 vs Control
Fig. 7. Hypothalamic TH activity at 5 and 15 min after intraventricular injection of (0.1 or 4 µmol) GABA.
Fig. 8. Effect of A0AA (0.46 mmol/kg bwt, ip) on plasma gonadotropin levels in OVX rats.
Fig. 9. Plasma PRL levels and hypothalamic TH activity following AOAA (0.46 mmol/kg bwt).

*P<0.05 vs Control.
endogenous brain GABA levels in earlier studies (Wood and Peesker, 1973), had no effect on plasma gonadotropin levels at 1h (Fig. 8).

Plasma prolactin concentrations were significantly reduced ($P<0.05$) at 1h following the injection of Amino-oxyacetic acid (Fig. 9).

**Hypothalamic TH activity:** Amino-oxyacetic acid (0.46 mmol/kg bwt.) significantly elevated hypothalamic TH activity at 1h ($P<0.05$) (Fig. 9).

**DISCUSSION**

The present results indicate that intraventricular injection of higher dose of GABA stimulates LH release. However, both doses of GABA failed to alter FSH release. GABA produced opposite effects on plasma PRL levels depending on the dose but there was no significant alteration of TH activity after either dose of GABA. Negro-vilar et al. (1980) reported a significant elevation in dopamine content in the median eminence following intraventricular injection of GABA, while there was no change in DA content of MBH and POA. The increased DA content in the median eminence noticed by Negro-Vilar et al. (1980) could possibly be due to the release of dopamine from the nerve terminals in ME since
TH activity in hypothalamus was not modified. This apparently indicates the failure of GABA to induce de novo synthesis of dopamine in the hypothalamus. Some of the GABA agonists also have been found to alter dopamine turnover in the hypothalamus without altering TH activity (Alderman and Shellenberger, 1974 and Lakti and Losey, 1974). An increase in hypothalamic LHRH levels after higher dose of GABA (Negro-Vilar et al., 1980) resulted in elevation of LH levels.

Aminoxyacetic acid induced suppression of PRL release and increased hypothalamic TH activity but had no effect on gonadotropin levels. It has been observed that castration suppresses GABA levels in different areas of brain and AOAA has been found to induce only a moderate increase in brain GABA levels in castrated rats (Earley and Leonard, 1973). The inhibition of pituitary PRL release by AOAA could be due to the failure of this compound to elevate endogenous brain GABA levels to the extent of 4 μmol, since the exogenous GABA at this higher dose significantly increased PRL levels. Aminoxyacetic acid, on the other hand, appears to have induced an elevation in brain GABA levels, that could still decrease plasma PRL concentrations. Though AOAA (6 mg/rat iv infusion) failed to affect PRL levels in castrated male rats, it inhibited sulpiride induced rise in plasma PRL (Debeljuk et al., 1980).
The inhibitory action of GABA on PRL release may be mediated through hypothalamus, since very small amounts of intraventricularly administered drugs reach the pituitary (Ojeda et al., 1978) and also intrapituitary injection of GABA has been reported to be ineffective in altering plasma PRL (Ondo and Pass, 1976). GABA agonist muscimol inhibits PRL secretion at relatively very low doses (Vijayan and Wuttke, unpublished observations, 1982). The increase in PRL levels after 4 μmol dose of GABA appears possibly due to a direct action of this transmitter on pituitary lactotrophs. Presence of GABA receptors in the pituitary supports the evidence for a direct action (Grandison and Guidotti, 1979; Grandison, 1981). When dopaminergic receptors were blocked with pimozide, plasma PRL was lowered instead of elevated by intraventricular GABA (Vijayan and McCann, 1978) and also intraventricularly injected muscimol reduced anterior pituitary DA concentrations, suggesting that the elevation in PRL could result from inhibition of dopaminergic tone (Casanueva et al., 1981). On the other hand, the higher dose of GABA could inhibit non-dopaminergic prolactin inhibiting factor (PIF) or stimulate the release of prolactin releasing factor (PRF) that could stimulate the release of PRL. Acute ether stress in pimozide pretreated rats consistently raised the levels of plasma PRL suggesting that the inhibitory factors are not involved since their receptors were blocked and that a
physiological releasing hormone must stimulate the release of PRL (Shin, 1980).

The organization of GABAergic pathways in the hypothalamus provides neuroanatomical evidence for an action of GABA exerted directly at pituitary level. In addition to hypothalamic GABAergic fibers projecting to the MBH, there is evidence for the existence of a medial basal hypothalamic tuberoinfundibular gabaergic pathway which project to the median eminence (Tappaz and Brownstein, 1977). Intraventricular injection of ethanolamine-o-sulfate (EOS) resulted in an increase in GABA content at pituitary level, attributed to inhibition of GABA-T in this pathway and not from blockade of GABA-T in the pituitary gland (Racagni et al, 1979). Gamma aminobutyric acid once released into the portal circulation could either inhibit or stimulate PRL release.

Regarding the stimulation of LH release, the physiological significance of GABA is still questionable since the dose required to obtain this response is rather large and also since GABA antagonist, bicuculline, while blocking the response to exogenous GABA, had varying effects on plasma LH levels (Vijayan and McCann, 1978). Aminoxyacetic acid also failed to influence plasma gonadotropin levels, while increasing brain GABA levels.