It is now clear that acetylcholine is the chemical mediator at all synapses between preganglionic and post-ganglionic fibers of the autonomic nervous system as well as at the myoneural junctions and at all postganglionic parasympathetic and some postganglionic sympathetic endings. The nerve impulse arriving at the end of the motor neuron evokes liberation of acetylcholine from the vesicles in the synaptic terminals. Fibers utilizing acetylcholine as mediator are referred to as cholinergic.

Acetylcholine is formed by the acetylation of choline by acetyl COA in the presence of an enzyme, choline
Acetylcholine esterase is an enzyme present in many tissues which hydrolyzes acetylcholine to choline and acetic acid.

Presence of Acetylcholine in high concentrations in hypothalamus, an area which is intimately involved in the control of AP hormone release, and also in adjacent areas like median eminence is well established (Martin, 1976; Vizi and Palkovits, 1978). Some of the cholinergic agonists and antagonists have been reported to alter AP hormone release (Grandison et al., 1974; Bruni and Meites, 1978). Atropine, which blocks muscarinic cholinergic receptors is used as an antidote to the toxic effects of anticholinesterases.

The effects of intraventricular administration of acetylcholine and/or atropine sulfate, the muscarinic Ach receptor blocker, on gonadotropin and prolactin release in OVX, conscious rats, were evaluated. Hypothalamic TH activity was also evaluated to determine dopaminergic mediation, if any, in inducing alterations in AP hormone release. Acetylcholine can interact with dopaminergic systems elsewhere in the brain (Giorgisieff et al., 1977).

Experimental procedure: Rats were cannulated as described earlier (Chapter II). Acetylcholine bromide (Sigma Chemical
Co. USA, Lot. 46C.0287) and Atropine Sulfate (Sigma Chemical Co. USA, Lot. 39C.3884) were prepared freshly in 0.9% NaCl, adjusted to pH 6.8 and 3rd ventricular injections were performed as described in Chapter II. Ach (20 µg) or atropine (20 or 100 µg) was administered either alone or atropine followed by 20 µg Ach at 15 min after atropine injection in a volume of 2.5 µl. Controls received equal volume of saline. Rats were decapitated at 5 or 15 min following microinjection of Ach or atropine or 15 min after Ach administration in atropine pretreated group. Brains were quickly removed for the assay of hypothalamic TH activity. Plasma gonadotropin and PRL levels were measured by RIA.

RESULTS

Effect of intraventricular Ach and/or atropine on Gonadotropin levels: 3rd ventricular injection of 20 µg acetylcholine induced a significant (P<0.05) elevation in plasma LH levels at 15 min (Fig. 10). On the other hand, intraventricular injection of either dose (20 or 100 µg) of atropine produced significant (P<0.05) suppression of LH at 5 min, which persisted upto 15 min (Fig. 10). Intraventricular acetylcholine (20 µg) failed to modify plasma LH levels in animals which were pretreated with either 20 or 100 µg dose of atropine (Fig. 10).
There was no significant alteration in plasma FSH levels following third ventricular Ach (20 μg) or either dose (20 or 100 μg) of atropine at 5 or 15 min (Fig. 11). Acetylcholine also failed to modify FSH concentrations in atropine pretreated rats (Fig. 11).

**Plasma PRL levels following intraventricular injection of Ach or atropine**: Plasma PRL levels were significantly lowered ($P < 0.01$) at 5 and 15 min following Ach (Fig. 12). Either dose (20 or 100 μg) of intraventricular atropine also induced a significant ($P < 0.05$) suppression of plasma PRL. However, the reductions in PRL levels induced by atropine were much less than those induced by Ach (Fig. 12).

Acetylcholine (20 μg) further reduced the plasma PRL levels in rats which were pretreated with the 20 μg dose of atropine, but not after the 100 μg atropine (Fig. 12).

**Hypothalamic TH activity**: There was a significant ($P < 0.05$) elevation in hypothalamic TH activity at 15 min following intraventricular injection of 20 μg dose of Ach (Fig. 13). Atropine had no effect on hypothalamic TH activity at 5 or 15 min (Fig. 13). Intraventricular Ach also induced a significant ($P < 0.05$) increase in hypothalamic TH activity in rats pretreated with 20 μg dose of atropine but failed to modify the enzyme activity in animals pretreated with 100 μg atropine (Fig. 13).
Fig. 10. Plasma LH levels following third ventricular injection of 20 μg acetylcholine or 20 or 100 μg atropine alone and 20 μg Ach in atropine pretreated rats. In this and subsequent figures in this chapter the value corresponding control or 'C' represents mean ± SEM of the pooled control samples. Arrow indicates the time of Ach injection in atropine pretreated rats.

*P<0.05 vs Control.
Fig. 11. Plasma FSH levels after intraventricular acetylcholine (20 µg) or atropine (20 or 100 µg) alone and 20 µg Ach in atropine pretreated rats.
Fig. 12. Plasma prolactin levels at 5 and 15 min following third ventricular injection of 20 μg acetylcholine or atropine (20 or 100 μg) alone and 20 μg Ach in atropine pretreated rats.

*P<0.05
> vs Control
**P<0.01
Fig. 13. Hypothalamic TH activity after intraventricular acetylcholine (20 μg) or atropine (20 or 100 μg) alone and Ach (20 μg) in atropine pretreated rats.

*P<0.05 vs Control
DISCUSSION

Third ventricular injection of acetylcholine significantly elevated plasma LH levels and suppressed PRL but only slightly elevated FSH levels. Atropine, a muscarinic Ach receptor blocker, on the other hand, suppressed LH release. Acetylcholine, however, failed to modify LH levels in animals which were pretreated with atropine. It would appear from the present findings that Ach receptors have stimulatory effect on LHRH release from hypothalamus. The failure of Ach to significantly elevate FSH could be due to the lesser effect of LHRH on FSH release. It is also possible that FSH may be controlled by a separate FSH releasing factor (FSH-RF). Surprisingly atropine alone also induced a suppression of PRL levels. The magnitude of the reduction in PRL levels were, however, less than those induced by Ach alone. Acetylcholine did not further induce decrease in PRL levels in rats which were pretreated with higher dose of atropine. This may be due to the total blockade of muscarinic receptors by atropine. The increased hypothalamic TH activity following Ach indicates that Ach stimulated dopamine synthesis in that area, which in turn could inhibit PRL release. This is in agreement with earlier reports after electrophysiological studies which showed that muscarinic receptors facilitate dopamine release
perhaps at the level of neuronal cell bodies in medial basal hypothalamus and further suggested that the effects of cholinergic agents on PRL release may be mediated in part through a cholinergic-dopaminergic interaction (Perkins and Westfall, 1979). It has also been demonstrated that the inhibitory effects of acetylcholine and its muscarinic agonists can be prevented by blocking dopaminergic receptors with pimozide, a dopaminergic receptor blocker (Vijayan and McCann, 1980). It is puzzling that atropine itself lowered PRL, which may possibly be due to some non-specific actions on other neurotransmitter systems at the doses employed here. In a previous study atropine has been found to enhance morphine induced PRL release, though itself could not alter basal levels of PRL at a dose of 10 mg/kg bwt (Fanjul et al, 1981). Although it is evident from the present results that Ach acts on hypothalamus to induce LH release, it is possible that Ach can directly act on anterior pituitary through hypophyseal portal system, since high affinity cholinergic receptors are present in anterior pituitary (Mukherjee et al, 1980). Their role as muscarinic or nicotinic receptors was indicated by their interaction with various cholinergic agonists and antagonists. Direct inhibitory actions of cholinergic compounds on PRL release in isolated anterior pituitary cells have been reported. However, cholinergic drugs failed to
alter the release of LH in these studies (Mukherjee et al., 1980). Consequently, the pituitary cholinergic receptors may affect the release of PRL but not LH and FSH. Muscarinic antagonists like scopolamine can greatly enhance morphine induced PRL release indicating that cholinergic system is directly involved and may be an essential link in the inhibitory regulation of PRL release (Fanjul et al., 1981).