Roles of γ-Aminobutyric Acid and Serotonin in the Arcuate Nucleus in the Control of Prolactin and Luteinizing Hormone Secretion

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Summary The effects of implantation of γ-aminobutyric acid (GABA) and serotonin (5-HT) into the arcuate nucleus (ARC) on serum luteinizing hormone (LH) and prolactin (PRL) were examined in the conscious ovariectomized rat. Following implantation of 5-HT, but not GABA, serum PRL was significantly increased. While 5-HT did not affect pulsatile LH secretion, GABA significantly decreased the mean LH concentration and pulse frequency, but not amplitude.

Key words: arcuate nucleus, γ-aminobutyric acid, serotonin.

Evidence has been accumulating that both serotonin (5-HT) (SCHNEIDER and McCANN, 1970; KAMBERI et al., 1971; GALLO and MOBERG, 1977) and γ-aminobutyric acid (GABA) (VIJAYAN and McCANN, 1978; LAMBERTS et al., 1983; ONDO and DOM, 1986) might be involved in the regulation of luteinizing hormone (LH) and prolactin (PRL) secretion from the anterior pituitary of the rat. Although the effects of intracranial administration of 5-HT and GABA on LH and PRL are a subject of controversy and the mechanisms by which 5-HT and GABA affect hormone secretion are not well understood, some investigators (GALLO and MOBERG, 1977; ONDO and DOM, 1986) have postulated that the hypothalamic arcuate nucleus (ARC) is one of the effective sites of such mechanism. In support of this, we recently showed that, in the ARC of the rat, 5-HT and GABA had potent excitatory and inhibitory effects, respectively, on the excitability of antidromically identified neurons by electrically stimulating the median eminence (ME) (NISHIHARA et al., 1986). These neurons were presumed to be tuberoinfundibular (TI) neurons which release neurohormones from their nerve terminals in the ME. Thus, it is suggested that the ARC mediates the effects of 5-HT and GABA on LH and PRL secretion. In the present study, we implanted small doses of 5-HT and GABA in the ARC of
ovariectomized rats, and evaluated the acute effects of them on serum LH and PRL levels by frequent sampling.

Adult female Wistar rats weighing 210–390 g were ovariectomized 2–3 weeks before the experiments. A double-cannula assembly made of stainless steel tubes with an outer diameter of 0.65 and 0.35 mm was used for implantation of drugs. Three days before blood sampling, the outer cannula was stereotaxically implanted into the unilateral ARC and kept closed with a mandril. The inner cannula was loaded with GABA (Tokyo Kasei, Tokyo) and 5-HT creatinine sulfate (Sigma, St. Louis) by tapping on the drugs piled on a glass plate. The approximate doses of GABA and 5-HT loaded were 100 and 25 nmol, respectively. A blood sample (150 µl) was withdrawn through the indwelling atrial cannula without anesthesia at 6-min intervals for 3 h. The mandril was replaced by the inner cannula 1.5 h after the start of blood sampling. An empty cannula was inserted as a control. At the end of blood sampling, the animals received cardiac perfusion with 10% formalin and frozen sections of the brain were prepared for histological confirmation of implantation sites. Animals having the implantation site out of the ARC were excluded from the experiment.

Serum LH and PRL were measured by the double antibody radioimmunoassay method using materials supplied by NIADDK and were expressed in terms of NIH-LH-S1 and PRL-RP-I, respectively. The intra- and inter-assay CVs estimated at a mean LH level of 10.2 ± 0.3 (S.E.M., n = 30) ng/ml were 8.8 and 12.6%, respectively, and those estimated at a mean PRL level of 20.9 ± 0.7 (n = 30) ng/ml were 12.4 and 13.9%, respectively.

The effect of the drugs on serum PRL levels were judged by comparing mean concentrations of PRL before and after the implantation of drugs. Since serum LH levels appeared to fluctuate in a pulsatile manner, a pulse was defined according to the CV method described by Gallo (1981). The CV was determined in an ascending as well as a descending phase of every pulsatile charge. A defined LH pulse had both ascending and descending CVs greater than twice the intra-assay CV of the corresponding assay. Pulsatile LH release was characterized by an amplitude (the difference between the peak and the preceding nadir), a frequency (number of pulses per 1.5 h before and after the insertion of the inner cannula) and the overall mean of the LH concentrations during a 1.5-h period. Differences between the values obtained before and after the implantation of drugs were statistically analyzed by Student’s t-test, and were considered to be significant at p < 0.05.

In the preimplantation period, although serum PRL occasionally appeared to fluctuate synchronously with serum LH, the fluctuation was not pulsatile as observed in the case of LH, i.e., the CV of serum PRL values composing each peak seldom exceeded twice the intra-assay CV for the corresponding assay. The means of serum LH and PRL for each experimental group are shown in Figs. 1 and 2, respectively. Implantation of GABA in the ARC resulted in a significant decrease in the mean LH concentration (Table 1). The LH pulse frequency, but not amplitude, was also significantly decreased, indicating that the decrease in the mean LH
concentration was mainly due to the decrease in the frequency, but not amplitude, of the LH pulse. Implantation of a 5-HT-containing or an empty cannula did not affect the mean LH concentration, LH pulse frequency or the amplitude.

Serum PRL concentrations were markedly, but transiently, increased soon after the implantation of 5-HT in the ARC. The mean serum concentration of PRL in the post-implantation period was significantly higher than that in the pre-

Fig. 1. Means of serum LH in animals implanted with empty (n = 5), GABA- (n = 7), and 5-HT-containing (n = 5) cannulae. Arrows indicate the time of implantation. Vertical lines represent S.E.M.
Implantation of a GABA-containing or empty cannula did not affect the mean serum concentration of PRL. Intraventricular injection of GABA or implantation of muscimol, a potent GABA agonist, into the medial preoptic-anterior hypothalamic area was shown to reduce LH secretion (LAMBERTS et al., 1983), although opposite results were also reported (PASS and ONDO, 1977; VIJAYAN and MCCANN, 1978). The present study

Fig. 2. Means of serum PRL in animals implanted with empty (n = 5), GABA- (n = 6), and 5-HT-containing (n = 5) cannulae. Arrows indicate the time of implantation. Vertical lines represent S.E.M.
first demonstrated that GABA could reduce pulsatile LH secretion by acting on ARC neurons. We have recently shown that GABA inhibited spontaneous activity of most of the ARC neurons (NISHIHARA et al., 1986). Since rat ARC is poor in LH-releasing hormone (LHRH) cell bodies (KAWANO and DAiKOKU, 1981), the inhibitory action of GABA on LH secretion might be mediated by ARC neurons other than LHRH neurons.

It has been reported that injection of relatively large doses of GABA (1,000–8,000 nmol) or muscimol (0.1–5 nmol) into either the medial basal hypothalamus or the ventricle stimulate PRL release, while smaller doses (100–2,000 nmol of GABA or 0.05 nmol of muscimol) have no effect (VIJAYAN and McCANN, 1978; ONDO and DOM, 1986; WILLOUGHBY et al., 1986). In agreement with this, 100 nmol of GABA did not affect the serum PRL concentration in the present study, suggesting that neurons in the ARC controlling PRL secretion are less sensitive to GABA than those controlling LH secretion.

It has been shown that intraventricular administration of 5-HT (KAMBERI et al., 1971) or treatment with p-chloroamphetamine (VAN DE KAR and BETHEA, 1982), a 5-HT-releasing drug, stimulates PRL secretion, though the precise site(s) at which 5-HT neurons control PRL secretion is currently not well understood. The present

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study strongly suggests that 5-HT stimulation of PRL secretion is, at least partially, mediated via the ARC. We previously showed that 5-HT facilitated and inhibited spontaneous activity of 49 and 23%, respectively, of presumed TI neurons in the ARC (Nishihara et al., 1986). It is possible that 5-HT directly inhibits the excitability of TI dopaminergic neurons which release dopamine as a PRL release-inhibiting hormone.

Contrary to the present results, intraventricular administration of 5-HT (about 6.5–13 nmol) has been shown to decrease serum LH (Kamberi et al., 1970; Schneider and McCann, 1970). The ineffectiveness of 5-HT in the present study cannot be attributed to the insufficiency of the dose used, since we implanted 25 nmol of 5-HT. It is probable, then, that the inhibitory effect of 5-HT seen in previous studies might be mediated by neurons in brain regions other than the ARC.

REFERENCES


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