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Hypothalamic tyrosine hydroxylase activity, plasma gonadotropin and prolactin levels after aminooxyacetic acid in ovariectomized rats[1]

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Summary. Plasma concentrations of gonadotropin, prolactin and hypothalamic tyrosine hydroxylase (TH) activity were measured in ovariectomized rats treated with aminooxyacetic acid (AOAA), a drug which elevates brain GABA levels. Hypothalamic TH activity was significantly increased with a significant decrease in prolactin (Prl) release. Plasma levels of gonadotropins were not modified by AOAA. These results support an inhibitory action of GABA on Prl release possibly mediated through hypothalamic dopamine.

Key words. Rat, ovariectomized; ovariectomy; aminooxyacetic acid; tyrosine hydroxylase, hypothalamic; gonadotropin, plasma; prolactin, plasma.

Gamma aminobutyric acid (GABA), an inhibitory synaptic transmitter in the central nervous system (CNS)[3], is involved in the control of anterior pituitary (AP) hormone release[4–6]. Both inhibitory and stimulatory actions of GABA on prolactin (Prl) release have been reported[7,8]. Aminooxyacetic acid (AOAA) elevates GABA levels in the CNS by inhibiting GABA-transaminase[9]. The present report demonstrates the role of endogenous GABA on gonadotropin and prolactin (Prl) levels after the administration of AOAA in ovariectomized (OVX) rats. Since dopamine (DA) is known to be a physiological inhibitor of pituitary Prl secretion, the activity of hypothalamic tyrosine hydroxylase (TH), the rate limiting enzyme in catecholamine biosynthesis, whose activity changes correlate well with presynaptic DA levels, was evaluated to investigate the dopaminergic mediation of GABA action.

Materials and methods. Ovariectomized adult female rats of Wistar strain were used 2–3 weeks after surgery. Aminooxyacetic acid was freshly prepared in 0.9% NaCl (pH adjusted to 7.0) and administered i.p. at a dose of 0.46 mmol/kg b.wt, in a volume equivalent to 1% of the b.wt. This dose of AOAA has been shown to produce a linear increase in GABA over the first 1-h period[9]. Controls received an equal volume of saline. Animals were decapitated after 1 h. Trunk blood was collected for gonadotropin and Prl measurement by a double antibody radioimmunoassay (RIA) using kits supplied by NIAMDD-NIH, Bethesda, USA. Results were expressed in terms of LH-RP-1, FSH-RP-1 and Prl-RP-2, respectively. The detection limits for the assay were 5 ng LH, 10 ng FSH and 0.25 ng Prl. The inter- and intra-assay co-efficients of variation were 10 and 6% for LH, 9 and 5% for FSH and 10.4 and 5.5% for Prl. Brains were removed and hypothalami (which included pre-optic area, medial basal hypothalamus and median eminence) were dissected out as described earlier[10], and TH activity was estimated according to the method of Shiman et al.[11].

Results. Saline injection did not alter gonadotropin, Prl levels or hypothalamic TH activity. Plasma gonadotropin levels were not modified by 0.46 mmol AOAA (fig. 1). The choice of this dose was based on earlier experiments where 0.46 mmol AOAA produced a maximal increase in endogenous GABA levels at 1 h[9]. Hypothalamic TH activity was significantly ($P < 0.05$) increased with a simultaneous decrease of Prl levels in plasma following the administration of AOAA (fig. 2).

Discussion. These results demonstrate that endogenous elevation of GABA by AOAA stimulates hypothalamic TH activity, resulting in the inhibition of Prl release. Earlier studies have

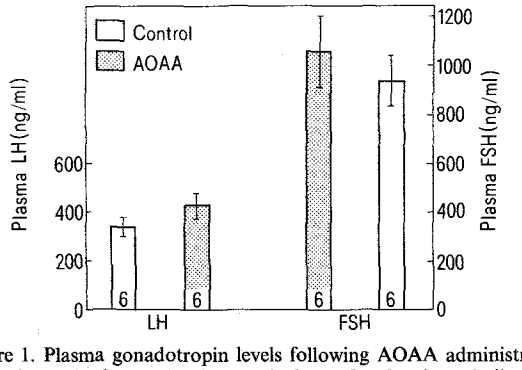

Figure 1. Plasma gonadotropin levels following AOAA administration in ovariectomized rats. Numbers at the base of each column indicate the number of animals in each group. Vertical lines represent SEM.

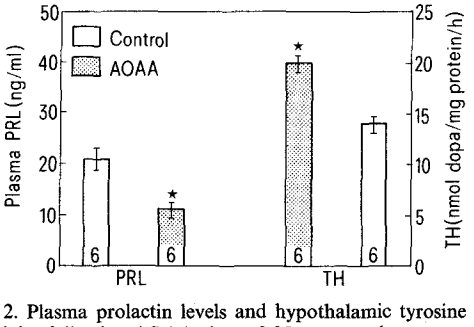

Figure 2. Plasma prolactin levels and hypothalamic tyrosine hydroxylase activity following AOAA. *$p < 0.05$ vs control.

shown that a dose of 0.1 µmol intraventricular GABA inhibited Prl release whereas a 4-µmol dose elevated Prl levels[6,8,12]. Castration reduced GABA levels in different brain regions and AOAA produced only a moderate increase of GABA in these rats[13]. The inhibition of Prl release by AOAA could possibly be due to the failure of this compound at the dose used here to elevate endogenous brain GABA levels to the extent of 4 µmol to produce an increase in Prl release. On the other hand, this dose of AOAA has induced an increase in the level of GABA that is capable of inhibiting Prl release. Intravenous infusion of AOAA failed to affect Prl levels in castrated male rats, but inhibited a sulpiride-induced rise in Prl[14].

The increased TH activity indicates that GABA concentrations induced by AOAA at this dose can stimulate de novo synthesis of hypothalamic dopamine (DA), which can be released from tubero-infundibular dopaminergic nerve terminals located in the median eminence into the hypophyseal portal blood and directly inhibit AP Prl release. This is supported by the fact that DA levels in hypophyseal portal blood are sufficiently high to have a direct action on pituitary to inhibit Prl release[15]. Aminooxyacetic acid is also shown to increase DA levels in nigro-striatal dopaminergic neurons[16]. A direct action of GABA on Prl release is possible since GABA receptors are located in the pituitary gland[17]. Aminooxyacetic acid failed to affect LH and FSH levels, and only very high doses of GABA resulted in stimulation of LH release[8]. The present observation supports the hypothesis that GABA is involved in the regulation of Prl release and appears to act through hypothalamic dopamine.

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Neuroendocrine control of carbohydrate metabolism in the freshwater bivalve mollusc *Lamellidens marginalis*

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Summary. Blood sugars and foot muscle glycogen were measured in the mussel, *L. marginalis* after ablation of the cerebral ganglia, and in mussels injected with cerebral ganglionic extract 3 h after ablation. There is a rise in the blood sugar and decrease in foot muscle glycogen 3 h after operation, but no change in sham-operated controls. The effect of ablation is reversed by injecting brain extract into ablated mussels. No such effect could be seen in the controls. The results are suggestive of the presence, in the cerebral ganglia, of a hypoglycaemic factor similar to insulin.
Key words. Mussel; *Lamellidens marginalis;* carbohydrate metabolism, neuro-endocrine control of; foot muscle glycogen; blood sugar; glycogen, foot muscle; hypoglycemic factor.

The neuroendocrine regulation of metabolism in pelecypod molluscs has received scant attention. The presence of neurosecretory cells in the central ganglia of a few bivalves has been reported[1-3], but their involvement in metabolic regulation is poorly understood. A cardioexcitatory neuropeptide has been isolated from the central ganglia of a bivalve[4], and the gut epithelium and hepatopancreas of some bivalves have been implicated in the production of insulin-like substances and control of carbohydrate metabolism[5,6]. However, nothing is known about metabolic regulation by the central ganglia of bivalves.

The present work is a report on the role of the cerebral ganglia of *L. marginalis* in regulating carbohydrate levels in the hemolymph and foot muscle.

Materials and methods. The mussels, *L. marginalis* of uniform size collected from ponds in Anantapur district were maintained in aquaria. After careful removal of one of the shell valves, the mussels were maintained for one week prior to experimentation. Ciliary activity of ctenidia and rate of heart beat were taken as indices of the health of the mussels after shell valve removal. The ablation of cerebral ganglia was done according to the method given by Nagabhushanam[7]. Control mussels were sham-operated in such a way that cerebral ganglia remained intact. A buffer extract (phosphate buffer pH 7.4) of cerebral ganglia from 15 mussels was prepared in a final volume of 3.0 ml. 0.1 ml of the extract was injected into the foot muscle of each mussel 3 h after ablation. Two sets of ablated mussels received separately 0.1 ml of phosphate buffer (pH 7.4) and 0.1 ml foot muscle extract prepared in phosphate buffer (pH 7.4), and served as controls. The sham-operated mussels were grouped into three sets and received separately brain extract, phosphate buffer (pH 7.4) and foot muscle extract.

Hemolymph was collected from the heart 1 h, 2 h, and 3 h after ablation and 30 min and 1 h after injection, as well as from normal unoperated mussels, centrifuged at 3000 rpm for 10 min to remove cellular elements, and total sugars were determined as total anthrone positive substances[8]. Foot muscle was isolated from each mussel and dried in a hot-air oven at 80 °C to constant weight, and glycogen was determined by the anthrone method[9]. The data were subject to statistical validation using Student's t-test for significance[10].

Results. There was a rise in blood sugar and decrease in foot muscle glycogen during 3 h following ablation of cerebral gan-