levels; $p < 0.001$ vs day values). By 2 h after the onset of light, pineal NAT activity in intact rats had returned to levels normally measured during periods of light. Bilateral SCGX completely abolished the nighttime rise in pineal NAT activity (figure 1). Unilateral SCGX rats had nighttime NAT values intermediate between those of intact or bilaterally SCGX rats. Pineal NAT activity in unilaterally SCGX rats increased to 20 and 37 times daytime values at 24.00 and 04.00 h, respectively. The 04.00 value in the unilaterally SCGX rats was lower ($p < 0.02$) than that in the intact rats and higher ($p < 0.001$) than that of rats lacking both their superior cervical ganglia.

Experiment 2. As with the NAT levels, pineal melatonin content in intact rats rose dramatically during the night; at 24.00 h the melatonin content was 9.5 times greater than daytime values while at 04.00 h this increased to 15.4 times higher than the mean values during the period of light (figure 2). Removal of both superior cervical ganglia completely prevented the nocturnal rise in melatonin while unilateral SCGX caused the nighttime values of melatonin to be at an intermediate level. Hence, at both 24.00 and 04.00 h the pineal melatonin content of the unilaterally SCGX rats was significantly lower ($p < 0.001$) than that of intact animals but significantly higher ($p < 0.001$) than similar values in the bilaterally SCGX rats.

*Discussion.* The nocturnal rise in pineal NAT activity in sham-operated rats reported in this study is comparable to that observed in similar experiments by other workers[5,7,12]. Usually the magnitude of the increase is on the order of 30–70-fold; in the present experiment NAT activity was 58 times greater at 04.00 h (during darkness) than during the day. The rise in pineal melatonin content during darkness also has been reported previously[6,13]. A strong correlation has been shown to exist between the nighttime rise in NAT activity and the increased content of melatonin[6]. Although in the present study NAT and melatonin were not assayed in the same glands, the observed results support a relationship between NAT activity and melatonin production.

Sympathetic denervation of the pineal gland (by removal of the superior cervical ganglia) has repeatedly been shown to negate the ability of the pineal to influence other endocrine organs[14,15]. As observed here, acute bilateral SCGX also is associated with an absence of a nocturnal rise in either NAT activity or melatonin during the second night following the operation. This is likely due to the loss of the neurotransmitter norepinephrine from the nerve endings within the pineal gland which follows interruption of the postganglionic sympathetic fibres to the pineal[16]. Unilateral SCGX curtailed the nighttime rises in both NAT activity and melatonin content. However, in contrast to the effects of bilateral SCGX, the increases in these parameters were not completely prevented by unilateral SCGX. It appears that unilateral SCGX impairs the ability of only a portion of the pinealocytes, the supposed functional elements of the pineal, to respond to darkness. It has not been determined whether long-term unilateral SCGX would also reduce, by an equivalent proportion, the ability of the pineal to suppress endocrine functions.

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## The involvement of serotonin in induced ovulation in the immature rat

V.D. Parker, K.F.A. Soliman[1] and C.A. Walker

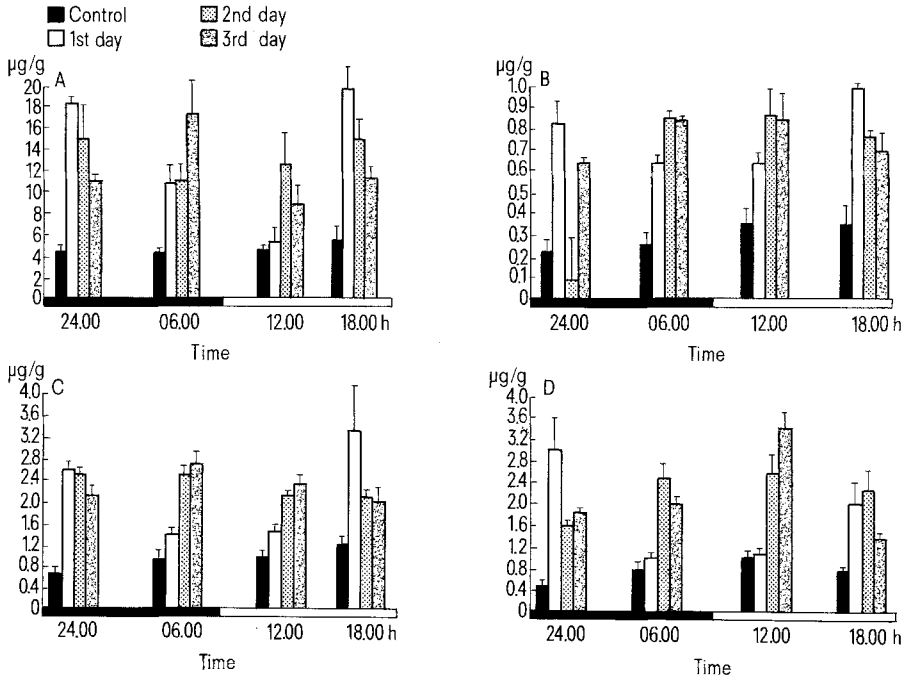
*School of Pharmacy, Florida A and M University, Tallahassee (Florida 32307, USA), 7 August 1978*

*Summary.* In pregnant mare's serum gonadotropin (PMS) treated immature rats the cortex, cerebellum, caudate nucleus and hypothalamus were isolated and analyzed for their serotonin (5-HT) content at 6-h intervals for 72 h. Results showed a general trend of significant variation occurring in days 1 and 3 after PMS injection with no major variations observed on the second day. The results obtained suggest a possible involvement of 5-HT in the control of ovulation.

A considerable body of literature supports the concept of serotoninergic interference with ovulation in rats[2–5]. Pregnant mare's serum gonadotropin (PMS) treated immature female rats given varied doses of serotonin (5-hydroxytryptamine, 5-HT) have been shown to produce a much smaller number of ova when compared with those recovered from control rats[2].

Other studies associated this interference with an inhibitory effort of 5-HT on certain hypothalamic catecholaminergic neurons that are normally responsible for facilitating ovulation[6–8]. Although many attempts have been made to correlate this inhibition on ovarian activity with endogenous 5-HT, few if any investigators have observed actual levels of the compound in the brain during the period leading up to ovulation. It was of interest, therefore, to examine 5-HT levels in the brain at different intervals during the initial ovulatory period in immature rats.

*Methods.* 24-day-old female rats of the Sprague-Dawley strain (Southern Animal Farm, Alabama), weighing 50–70 g, were maintained at a constant temperature (22±1 °C) in a light-controlled room (light on from 09.00 to 21.00 h). Animals were provided with standard Purina Lab Chow and water ad libitum. After a period of 4 days, 36 rats were injected s.c. with saline solution containing 25 IU PMS




Effect of PMS treatment on 5-HT levels in *A* caudate nucleus, *B* cerebellum, *C* cerebral cortex and *D* hypothalamus. Each column represent the average of 6 animals ±SEM. 5-HT concentration was based on brain wet weight.

(sigma). Animals were sacrificed every 6 h by decapitation, and the entire brain was removed and rapidly separated into 4 parts: cerebral cortex, cerebellum, caudate nucleus and hypothalamus. The brain tissues were immediately frozen in a super histofreeze and stored until needed for the analysis. 5.HT was extracted and the levels were determined using the method of Welch and Welch[9]. The Amin-co-Bowman Spectrophotofluorometer was used at a wavelength of 385 nm for activation peak and 490 nm for emission. Data was subjected to analysis of varience using F-test.

*Results.* The figure, A, shows the levels of 5-HT in the caudate nucleus in control and treated animals. In this figure it is clear that PMS administration to the animals caused a significant increase ($p < 0.05$) in the 5-HT content which persist throughout the 3 days of study. In the control animals there was no significant rhythm detected, although the levels were minimum during the dark phase. On the first day after PMS treatment, peak concentration occurred at 18.00 h during the light phase and trough values occurred at 12.00 h. Peak values for the second day were observed at 24 h in the dark phase. Third day determinations showed peak levels occurring at 06.00 h into the dark phase, and trough levels at 12.00 h in the light phase. Peak and trough differences were statistically significant ($p < 0.05$) on days 1 and 3, with no significant difference in values on day 2 ($p < 0.05$).

The figure, B, gives the data obtained for 5-HT levels in the cerebellum. The administration of PMS caused significant rise of 5-HT. In the control animals there was no significant variation in 5-HT contents. Peak levels for the first day were observed at 18.00 h into the light phase, with trough levels occurring at 06.00 h, in the dark phase. While differences in peak and trough values for the first day were statistically significant ($p < 0.05$), no significant difference in values for days 2 and 3 could be detected ($p < 0.05$). The diurnal variation of 5-HT concentration in the cortex in control and PMS treated animals is presented in the figure, C. Significant circadian diurnal rhythm was observed in control animals. There was an elevation in 5-HT cortical contents after PMS treatment. Due to PMS treatment the diurnal variation was reversed in the first day, however differences in peak and trough values in the cerebral cortex were not significantly different on the second or the third day (figure, C). On the first day, highest concentrations were reached at 18.00 h in the light phase, while lowest concentrations were observed at 12.00 h, also in the light phase. Highest values for the second day were obtained at 24.00 h dark phase, with lowest values occurring at 18.00 h light phase. The highest concentrations observed during the third day appeared to levels off between 06.00 and 12.00 h with a gradual decrease throughout the remainder of the day, reaching a trough value at 24.00 h in the dark phase.

The hypothalamic 5-HT contents is presented in the figure, D. Significant diurnal ($p < 0.05$) variations were noticed in the control group with minimum values during the dark phase. Hypothalamic concentrations of 5-HT throughout the 3 days period showed a slight shift in peak levels when compared to those found in the cerebellum and cerebral cortex. In the hypothalamus, peak values were observed at the end of the first day at 24.00 h in the dark phase of the period. Lowest levels were found earlier on the first day at 12.00 h in the light phase. No significant changes in 5-HT levels were observed during the second day. Third day determinations of showed the 5-HT peak levels occurred at 12.00 h in the light phase, with drop in concentration at 18.00 h, also in the light phase. The data presented here shows that PMS treatment results in an elevation of 5-HT level in all brain regions studied and daily rhythm of 5-HT levels existed in all tested brain regions in the control animals (minimum in the dark phase). This periodicity is converted in all brain regions tested at the first day after PMS treatment. During the second and third day after PMS injection there is an obvious trend towards the normal daily rhythm.

*Discussion.* The data presented here indicate that 5-HT levels are affected by PMS treatment, which suggests an influence of PMS on 5-HT synthesis, release and/or metabolism. The significant rise of 5-HT after the administration of PMS indicate that this biogenic amine is important for the ovulation process. The intervention of the 5-HT neurons systems in the overall regulation of cyclic LH release have been suggested. Blockade of 5-HT synthesis by para-chlorophenylalanine facilitates ovulation when the

drug is administered just before the 'critical period' in adult rat[10]. However, it has opposite effects when 5-HT synthesis is inhibited 20 h earliers[11], this seems to suggest that although 5-HT synaptic terminals located in the median eminence are involved in the preovulatory processing of information necessary for preparing the critical period.

More recently it was indicated that 5-HT administration does not inhibit ovulation at a central site but acts as peripheral vasoconstrictor preventing the passage of hormones to their target organs[12].

There is some supporting evidence for the results obtained here; treatment of ovariectomized rats with steroids increases hypothalamic tryptophan levels[13] and in the midbrain[14]. Significant ($p < 0.01$) decline in 5-HT level was noticed at 48 h after PMS injection which is presumably equivalent to the critical period for the release of LH. The results obtained here is also supported by the in vitro work which indicate that 5-HT can inhibit gonadotropin release from pituitaries but only in the presence of hypothalamic fragments. More over 5-HT has been shown to stimulate the release of prolactin[4].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## In vitro stimulation of chicken pituitary growth hormone and prolactin secretion by chicken hypothalamic extract


S. Harvey, C.G. Scanes, A. Chadwick and N.J. Bolton

*Department of Zoology, University of Hull, Hull HU6 7RX (England), Department of Physiology, Rutgers University, New Brunswick (New Jersey 08903, USA) and Department of Pure and Applied Zoology, University of Leeds, Leeds LS2 9JT (England), 12 September 1978*

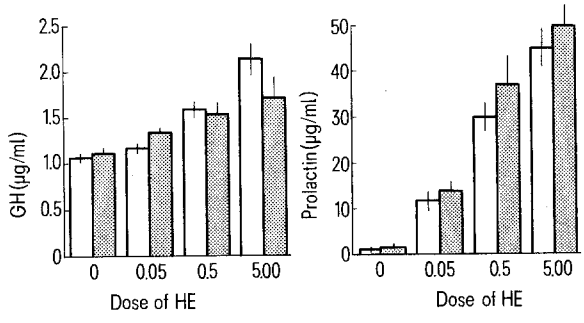


*Summary.* The effect of an acid extract of chicken hypothalami on the in vitro secretion of prolactin and growth hormone (GH) by dispersed chicken pituitary cells has been investigated. Both prolactin and GH release were stimulated in a dose related manner in the presence of the hypothalamic extract (HE). Somatostatin had no effect on the basal or HE stimulated release of prolactin although it did inhibit the HE induced release of GH.


The existence of growth hormone (GH) releasing activity in the avian hypothalamus has long been established[1-4]. However, this activity has so far only been demonstrated using bioassay[1,2] and electrophoretic[3,4] techniques to measure hypothalamic extract (HE) induced pituitary GH release. Therefore, in view of the fact that chicken HE has recently been found to have no stimulatory effect on the levels of immunoreactive plasma GH in male chickens[5,6], the effect of this extract on the in vitro release of immunoreactive pituitary GH has been investigated in the present study. In addition the effect of this extract on the in vitro secretion of immunoreactive prolactin has also been determined to confirm and extend other studies[7-11] which have established the prolactin releasing activity of avian HE. The influence of incubating somatostatin (growth hormone release inhibitory hormone, GHRIH) alone and together with chicken HE on prolactin and GH release has also been assessed; GHRIH having been found to inhibit in vitro GH release in avian[12] and mammalian[13,14] species and to inhibit prolactin release from incubated rat pituitary cells[15].

*Materials and methods.* Heads from 8-10-week-old broiler fowl were obtained from a local packing station and the adenohypophyses were removed and collected into ice cold medium 199 (Wellcome Laboratories Ltd) in which they were rapidly transported to the laboratory. Pituitary cells were prepared following the method of Bicknell and Follett[16]. The incubations were carried out in $6.3 \times 0.8$ cm disposable polystyrene tubes in a shaking water bath at 39 °C under an atmosphere of 95% $O_2$, 5% $CO_2$. To each tube a 0.5 pituitary equivalent was added in 0.5 ml of medium 199 and the test substance then added in a further 0.5 ml of media. After a 2-h incubation the cells were separated from the media by centrifugation (20 min at $1000 \times g$) and the supernatants stored deep frozen prior to assay.

An acid extract was prepared from broiler fowl hypothalami following the method of Follett[17] and was neutralized prior to use and diluted with medium 199. Linear GHRIH was obtained commercially (Digby Chemical Service). Control incubations were in medium 199 alone. Extracts of




Effect of chicken HE, alone (open columns) and in the presence of GHRIH (50 ng/ml; filled columns) on the in vitro release of prolactin and GH from dispersed chicken pituitary cells. Means±SEM (n=5).