## Prolactin Surge on the Afternoon of Pro-Oestrus in the Rat and its Blockade by Pentobarbitone

Prolactin (PL) content of the pituitary gland has been reported to be high in pro-oestrus and oestrus in the cycling rat[1,2]. KWA and VERHOFSTAD[3] found by radioimmunoassay that a surge of PL occurred on the afternoon of pro-oestrus in the rat. Using the same procedure, the highest PL levels in serum were reported to occur at oestrus by AMENOMORI, CHEN and MEITES[4], though the levels were not significantly higher than those at pro-oestrus or metoestrus.

It is known that LH content of the pituitary gland decreases on the afternoon of pro-oestrus[5-7], and it has been suggested that this decrease is triggered by an increase in ovarian oestrogen secretion[8,9]. Oestrogen is known to increase the pituitary and the serum levels of PL[10].

The present paper deals with changes of PL content of the pituitary during the oestrous cycle, with special reference to the critical period for LH release.

*Material and methods.* Female virgin rats of the Wistar-Imamichi strain (240–280 g) were used. They were maintained in a temperature controlled (24 ± 2 °C) and light controlled (on at 05.00 h, off at 19.00 h) animal room. Food (CA-1, Nihon CLEA Ltd., Tokyo) and water were given ad libitum. Vaginal smears were taken every morning between 09.30–10.00 h. Only animals showing at least 2 regular, consecutive 4-day-cycles were used. Animals were decapitated under light ether anaesthesia between 10.30 and 11.00 h or between 17.30 and 18.00 h on each day of the cycle. In some animals showing a pro-oestrous smear, sodium pentobarbitone (Nembutal, Abbott, 35 mg/kg body wt.) was injected i.p. at 13.30 h. The animals were killed between 17.30 and 18.00 h of the same day.

Anterior pituitary glands were removed, weighed and frozen at −20 °C until the time of assay. PL levels in the anterior pituitary gland were determined by disc electrophoresis on polyacrylamide gel and measured by a microdensitometer (Canalco, Model E)[11]. A linear relationship between the graded doses of the standard preparation of PL and their optical densities has already been reported by YANAI and NAGASAWA[11] and NICOLL, PARSONS, FIORINDO and NICHOLS[12].

*Results and discussion.* The changes in weight, PL content and concentration of the pituitary gland during the oestrous cycle are shown in the Table.

PL content and concentration attained their highest values in the afternoon of oestrus (E). The values declined slightly in the morning of the 1st day of di-oestrus ($D_1$) and remained at almost the same level during the afternoon of the same day. The content of PL in the morning of the 2nd day of di-oestrus ($D_2$) was significantly less ($P < 0.05$) than that in the afternoon of E, but not significantly different from that in the morning of E and $D_1$ and in the afternoon of $D_1$ and $D_2$. However, the amount of PL per unit weight of the anterior pituitary at this stage was not significantly different from all other stages described above. Either content or concentration of PL in the pituitary gland was slightly, but not significantly, higher in the afternoon than in the morning on each of the days E, $D_1$ and $D_2$.

The pituitary PL content on the morning of the day of pro-oestrus (PE) remained at the same level as observed on the morning of E or on the afternoon of $D_1$ and $D_2$. On the afternoon of PE, a significant decrease was found in PL content and concentration of the pituitary from the high level observed on the morning of PE ($P < 0.05$). The concentration of PL in the afternoon of PE was significantly less than that obtained in other stages of the cycle ($P < 0.05$). This decrease in PL concentration and content in the pituitary gland seems to coincide well


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Prolactin content and concentration of the anterior pituitary of rats at various stages of the oestrous cycle

| Stage of oestrous cycle | Time of autopsy | Treatment | No. of rats | Anterior pituitary (AP) weight (mg) | Prolactin content[a] ($cm^2$/AP) | Prolactin concentration[a] ($cm^2$/mg AP) |
|---|---|---|---|---|---|---|
| Oestrus | Morning (M) | – | 5 | 10.32 ± 0.45[b,e] | 19.34 ± 1.62[g,h] | 1.92 ± 0.11[k] |
| Oestrus | Afternoon (A) | – | 4 | 9.85 ± 0.51[e] | 24.48 ± 4.18[g] | 2.44 ± 0.35[k] |
| Di-oestrus 1[c] | M | – | 4 | 9.30 ± 0.48[e] | 17.74 ± 3.90[g,h] | 1.88 ± 0.36[k] |
| Di-oestrus 1 | A | – | 4 | 9.23 ± 0.43[e] | 19.50 ± 1.71[g,h] | 2.15 ± 0.27[k] |
| Di-oestrus 2[d] | M | – | 4 | 7.53 ± 0.33[f] | 12.37 ± 2.12[h,i,j] | 1.62 ± 0.23[k] |
| Di-oestrus 2 | A | – | 4 | 8.78 ± 0.23[e] | 18.00 ± 2.12[g,h] | 2.07 ± 0.30[k] |
| Pro-oestrus | M | – | 5 | 9.60 ± 0.36[e] | 16.23 ± 2.06[h,i] | 1.66 ± 0.17[k] |
| Pro-oestrus | A | – | 5 | 10.00 ± 0.40[e] | 5.58 ± 0.91[j] | 0.56 ± 0.10[l] |
| Pro-oestrus | A | Na-Pento-barbitone (35 mg/kg, ip.) | 5 | 9.24 ± 0.28[e] | 17.35 ± 2.36[g,h] | 1.90 ± 0.28[k] |

[a] Prolactin content and concentration are expressed as optical density ($cm^2$). [b] Mean ± S.E.M. [c] 1st day of di-oestrus. [d] 2nd day of di-oestrus. [e–l] Mean which have the same superscripts are not significantly different from each other at 5% level.

with the elevation of PL level of plasma reported by KWA and VERHOFSTAD[3].

The decrease in either PL content or concentration observed in the afternoon of PE was prevented by the injection of sodium pentobarbitone (Pb) at 13.30 h of PE. The level of PL in the pituitary of the Pb injected animals remained high even at 17.30 h, and it was significantly higher than corresponding values of intact animals.

These findings seem to indicate that the release of PL takes place on the afternoon of PE around the 'critical period' for LH surge, and that the central nervous system could trigger the release. NEILL[13] reported increase in plasma prolactin in the afternoon of proestrus in the rat and WUTTKE and MEITES[14] blocked the increase by pentobarbital.

*Résumé.* La teneur et la concentration de la prolactine dans le lobe antérieur de l'hypophyse ont été déterminées aux temps variés de l'oestrus chez la ratte. Dans l'après-midi du jour du pro-oestrus, la teneur et la concentration dans la glande s'abaissent. Ce changement a été bloqué par le pentobarbitrate de sodium injecté par voie i.p. á 13.30 h au jour du pro-oestrus.

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## The Effect of Acetylcholine on Adrenal Function in the Hypophysectomized Dog

Until recently, the significance of an intimate anatomic proximity of adrenal cortical to medullary tissue was obscure. In 1965, WURTMAN and AXELROD[1] reported the effect of adrenal glucocorticoids upon the medullary enzyme phenylethanolamine-N-methyl transferase (PNMT) which is responsible for the N-methylation of norepinephrine to form epinephrine. This enzyme is found to be localized in highest concentrations in the adrenal medulla of mammals.

Acetylcholine (ACh) has been shown to be the physiological stimulus for the release of epinephrine and norepinephrine from the adrenal medulla. However, the in vivo role of acetylcholine as an adrenal cortical stimulus has not been explored.

The main objective of the present investigation was to simultaneously study the acute and direct effect of ACh on adrenal medullary and cortical activity in the hypophysectomized animal.

*Experiments.* 4 mongrel dogs of both sexes weighing between 20 and 28 kg were used. Following the induction of anesthesia with 30 mg of i.v. sodium pentobarbital per kg of body weight, a maintenance airway was provided with the placement of a tracheal canula. Blood pressure was recorded from the right carotid artery by a transducer and physiograph instrument. A 0.9% sodium chloride i.v. drip was begun. The animals were then immediately hypophysectomized by the transbuccal approach as described by MARKOWITZ and ARCHIBALD[2]. 4 h later, the right adrenal was removed and quickly frozen using dry ice and acetone.

A teflon catheter, sized according to the dog's body length, was then filled with sodium heparin, and passed via the left femoral vein into the inferior vena cava, and thence into the left lumbar-adrenal vein and a securing ligature placed. This catheter allowed the direct collection of adrenal venous blood into heparinized tubes which were maintained in ice until centrifuged for 10 min at 2000*g*. The plasma was removed and stored at $-10\,^{\circ}C$ for analysis of catecholamine and hydrocortisone concentration.

At the conclusion of the experiment, the left adrenal gland was removed and quickly frozen with dry ice and acetone and stored at $-10\,^{\circ}C$ until analysis.

Acetylcholine was injected into the femoral vein in doses ranging from 0.42 and 0.95 mg/kg and blood samples were drawn 5 min after each injection.

The adrenal glands and adrenal venous blood were assayed for epinephrine and norepinephrine by the method of von EULER and LISHAJKO[3]. The adrenal glands were also assayed for PNMT activity by the method described by AXELROD[4] involving the use of $C^{14}$-S-adenosylmethionine. (One unit of enzyme activity

Table I. Dose-response to acetylcholine in hypophysectomized dogs

| Dog No. | ACh dose (mg/kg) | 170 HCS | Epi | Norepi | % E of total cat. secretion |
|---|---|---|---|---|---|
| 1 | 0 | | 5.0 | 12.6 | 35 |
| | 0.42 | 0 | 41.0 | 12.9 | 76 |
| | 0.83 | 0 | 171.1 | 55.8 | 76 |
| 2 | 0 | 0.4 | 4.2 | 8.9 | 31 |
| | 0.43 | 0.1 | 41.5 | 5.2 | 89 |
| | 0.72 | 0.0 | 119.4 | 10.9 | 90 |
| 3 | 0 | 0 | 2.8 | 3.0 | 48 |
| | 0.5 | 0 | 76.3 | 18.1 | 81 |
| 4 | 0 | 0.2 | 3.4 | 10.5 | 25 |
| | 0.48 | 0.9 | 60.0 | 14.2 | 80 |
| | 0.72 | 0.1 | 180.6 | 36.8 | 81 |
| | 0.95 | 0 | 225.0 | 45.3 | 83 |

170 HCS reported as μg/ml of plasma. Epinephrine and Norepinephrine reported as μg/100 ml plasma.

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