A biphasic effect of estradiol on serotonin metabolism in rat pineal organ cultures[1]

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Summary. Physiological amounts of estradiol (1 nM) significantly increased serotonin conversion to melatonin and 5-methoxytryptophol by rat pineal glands in organ culture whereas larger, pharmacological, doses (i.e. 1000 nM) impaired it significantly. The stimulatory but not the inhibitory effect of estradiol was blocked by the simultaneous addition of puromycin in the culture medium.

Estradiol treatment has been shown to affect various aspects of pineal metabolism, including size and matrix of pineal cell mitochondria, synthesis and content of nucleic acids, proteins and lipids, serotonin and norepinephrine turnover rates, progesterone metabolism, and norepinephrine-induced increase of adenylcyclase activity and of pineal membrane potential (for references see Cardinali[2]). These effects depend partially upon direct hormonal action since cytoplasmic and nuclear receptors for estradiol are present in pineal cells from various species[3], and since physiological amounts of estradiol (i.e. 1-15 nM, in the concentration range of the K_d for cytosol and nuclear binding sites) increase in vitro melatonin synthesis[3,4], DNA-dependent RNA polymerase activity[4] and the synthesis of a specific estradiol-induced protein[5].

Estradiol treatment in vivo results in a biphasic, dose-related effect on the pineal melatonin-forming enzyme hydroxyindole-O-methyl transferase (HIOMT); low doses (0.05-1 µg/day) are stimulatory, whereas high doses (i.e. more than 5 µg/day) are inhibitory[2,6]. Since injection of 2 µg of estradiol in ovariectomized rats produced translocation of pineal receptor-hormone complexes from cytoplasm to nuclei indistinguishable from that found in normal rats at proestrus[2], 2 µg of estradiol should be taken as the highest dose to be injected daily to examine changes within the physiological range. In order to examine whether the same biphasic effect of the hormone occurs in vitro, rat pineal organ cultures were used.

Material and methods. Pineal glands obtained from adult Wistar female rats castrated 3 weeks earlier were incubated under sterile conditions in 0.2 ml of TC 199 medium at 37 °C for 24 h as described elsewhere[2]; the animals were killed at 10.00 h. 2 studies were carried out: In a 1st experiment 5 pineal glands per flask were incubated with 2 µCi of [^{14}C] serotonin binoxalate (15.5 Ci/mole, New England Nuclear Co. Boston, MA) in the presence of none, 1 nM or 1000 nM estradiol. A blank containing no tissue was included in each series of incubations. Reactions were stopped by separating the media from the pineal glands. Tissues were blotted dry and weighed; aliquots of the incubation media (20 µl) plus 20 µl of a mixture (4 µg each) of authentic serotonin, 5-methoxyserotonin, 5-hydroxyindoleacetic acid, 5-methoxyindoleacetic acid, 5-hydroxytryptophol, 5-methoxytryptophol, N-acetylserotonin and melatonin, were chromatographed immediately in duplicate by 2-dimensional TLC developed with: A) chloroform-methanol-glacial acetic acid (93:7:1, by vol.; 2 runs) and B) ethyl acetate[7]. TLC was performed in darkness and under an atmosphere of N_2. The compounds were visualized using the Prochazka reagent and the UV positive zones were scraped into a counting vial. [^{14}C] Radioactivity was determined by liquid scintillation spectrometry after adding 1 ml

Table 1. Effect of estradiol on serotonin metabolism by rat pineal organ cultures

	Control	Estradiol 1 nM	1000 nM
Origin (serotonin, 5-methoxyserotonin)	51,010± 14,120	50,550± 10,742	51,340± 11,972
5-Hydroxyindoleacetic acid	3,236± 1,123	3,962± 1,986	3,292± 789
5-Methoxyindoleacetic acid	106± 37	134± 40	78± 27
5-Hydroxytryptophol	206± 41	240± 67	223± 34
5-Methoxytryptophol	106± 21	190± 29*	50± 9*
N-Acetylserotonin	658± 197	597± 209	575± 92
Melatonin	474± 80	697± 108*	223± 40*

Pools of 5 pineal glands were incubated as described in the text. Portions of the incubation media (402,000± 15,000 dpm) were chromatographed by TLC. Results are expressed as dpm/mg of tissue, mean± SD (n= 5 in each group). * Significantly different from control, $p < 0.05$, Dunnet's t-test.

Table 2. Effect of estradiol on serotonin metabolism by rat pineal organ cultures in the presence of puromycin (0.2 mg/ml)

	Control	Estradiol 1 nM + puromycin	Estradiol 1000 nM + puromycin	Puromycin
Origin (serotonin; 5-methoxyserotonin)	52,017± 12,905	51,766± 14,094	52,257± 11,653	51,886± 15,576
5-Hydroxyindoleacetic acid	3,537± 856	3,794± 920	3,651± 1,048	4,000± 1,316
5-Methoxyindoleacetic acid	126± 13	137± 25	91± 25	74± 25
5-Hydroxytryptophol	200± 25	177± 51	188± 51	154± 38
5-Methoxytryptophol	108± 13	126± 13	57± 25*	97± 13
N-Acetylserotonin	629± 127	571± 25	697± 153	486± 128
Melatonin	526± 126	573± 166	200± 51*	446± 102

Pools of 5 pineal glands were incubated as described in the text. Portions of the incubation media (398,000± 20,000 dpm) were chromatographed by TLC. Results are expressed as dpm/mg of tissue, mean± SD (n= 5 in each group). * Significantly different from the other groups, $p < 0.05$, analysis of variance, Tukey's test.

ethanol and 10 ml toluene phosphor. Results were expressed as dpm/mg of tissue in the corresponding chromatographic zone; differences between duplicates were less than 15%. Except for the origin, each value was corrected for the radioactivity of the blank. Blank radioactivities expressed as dpm were: 5-hydroxyindoleacetic acid: 105 ± 20; 5-methoxyindoleacetic acid: 30 ± 10; 5-hydroxytryptophol: 25 ± 8; 5-methoxytryptophol: 29 ± 7; N-acetylserotonin: 25 ± 6; melatonin: 30 ± 10. Validation of this procedure by further TLC in various solvent system was published elsewhere[8]. In a 2nd experiment, 5 pineal glands per flask were incubated as above in the presence of none, 1 or 1000 nM estradiol and of 0.2 mg/ml puromycin. The conversion of ^{14}C serotonin to hydroxy- and methoxyindoles was determined as in experiment 1.

Results. As described previously[2] estradiol in the nmolar range enhanced serotonin conversion to 5-methoxy derivatives, particularly 5-methoxytryptophol and melatonin (table 1). In contrast concentrations of estradiol 1000 times greater decreased serotonin metabolism to both derivatives ($p < 0.05$). The stimulatory but not the inhibitory effect of estradiol on the in vitro metabolism of serotonin by pineal glands was blocked by adding puromycin to the culture medium (table 2). Puromycin per se did not affect serotonin metabolism significantly in this preparation.

Discussion. Foregoing data indicate that, as in vivo[2,6], the in vitro activity of estradiol on rat pineal gland exhibits a biphasic, dose-related pattern. In physiological concentrations estradiol enhanced the conversion of serotonin to the hormonally-active O-methylated derivatives 5-methoxytryptophol and melatonin, whereas greater, pharmacological, amounts impaired that metabolic reaction. The stimulatory effect of estradiol observed at physiological concentrations was blocked by puromycin, supporting the view that the translocation of estradiol-receptor complexes from the cytoplasm to the nuclei observed in incubated pineals[2] eventually results in genomic activation of pineal cells. In contrast to the stimulation that followed low concentrations of estradiol in culture medium, the inhibition observed at high, pharmacological doses, was not affected by adding puromycin. Hence inhibition of 5-methoxyindole synthesis by a large dose of estradiol seems not to depend upon changes in protein synthesis. Recent observations on the O-methylation of estradiol to its 3-methyl-ether by a partially purified bovine pineal HIOMT offer a basis for interpreting our results[9]. Since HIOMT K_m values for estradiol and N-acetylserotonin were close, and since in the presence of estradiol the K_m for S-adenosylmethionine was about one order of magnitude lower than in the presence of N-acetylserotonin, the possibility that high doses of estradiol may inhibit O-methylation of 5-hydroxyindoles in vivo and in vitro in a competitive way should be considered.

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Effect of apomorphine hydrochloride administration on serum concentrations of prolactin and growth hormone in cattle

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Summary. Administration of apomorphine hydrochloride to cattle significantly depressed serum prolactin (PRL) concentrations and elevated serum growth hormone (GH) concentrations in a dosimetric fashion.

Prolactin (PRL) and growth hormone (GH) play important roles in regulating either the initiation or maintenance of lactation in cattle[1-3]. Growth hormone is also considered important in controlling growth processes of cattle[4]. Neuroendocrine mechanisms controlling pituitary hormone synthesis and release are obscure in ruminants, but have been extensively studied in laboratory animals. Dopamine (DA) and norepinephrine (NE) modulate secretion of GH[5] and PRL[6-10]. Studies with apomorphine hydrochloride, a selective DA receptor agonist, suggest that in humans, an inverse dopaminergic system exists controlling GH and PRL secretion[11,12]. The purpose of this investigation was to determine if such a system exists in cattle.

Materials and methods. 10 Holstein heifers (age 10–12 month) were used in this study. Body weights for animals ranged from 280 to 326 kg. Cattle were fitted with indwelling jugular cannulae and were allowed free choice to water during experimentation. Food was restricted 8 h prior to and during experimentation. On the day following cannulation, blood was collected and discarded for 2 h in order to accustom animals to experimental conditions. Blood samples were collected at 15 min intervals for 2 h prior to treatment (control). Immediately after the last sample was withdrawn, 1 ml of 0.1% sodium metabisulfite was administered over 1 min to all animals (control). Further blood samples were withdrawn at 5, 10, 15, 30, 45, 60, 75, and 90 min after sodium metabisulfite administration. After the last sample was withdrawn, cattle were divided into 5 groups, each consisting of 2 animals. Apomorphine hydrochloride (APM, dissolved in 0.1% sodium metabisulfite) was administered as described above. Cattle in group 1 received 0.01 µg/kg b.wt, group 2 received 0.1 µg/kg b.wt, group 3 received 1.0 µg/kg b.wt, group 4 received 10 µg/kg b.wt and group 5 received 100 µg/kg b.wt of APM. Blood was then collected at 5, 10, 15, 30, 45, 60, 90, 120 and 150 min after APM administration.

Bovine PRL was assayed by the method of Butler[13]. Serum GH was assayed using a double antibody radioimmunoassay, as described by Purchas et al.[14] with minor modifications. Bovine GH (NIH-GH-B18)[15] was used as the binding