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Pineal Compounds Alter Prolactin Release from Bovine Pituitary Cells[1] (40446)

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The photoperiod is an important regulator of prolactin (PRL) secretion in cattle (1). Since the pineal is the only gland whose activity has been biochemically (2) and physiologically (3) linked directly to changes in the photoperiod, its secretions may mediate the photoperiod influences on the secretion of pituitary hormones. Pinealectomy or constant light (4, 5) increased, whereas blinding (6) decreased pituitary prolaction concentration in rats. Our objective was to determine the effect of several compounds, normally found in the bovine pineal, on PRL release from bovine pituitary cells in culture. Our rationale was to identify pineal compounds with the potential to mediate photoperiod-induced changes in PRL secretion.

Material[3] *and methods.* Bovine pituitary cell cultures were prepared as previously described (7). Briefly, bovine anterior pituitaries were diced, and the cells were then dispersed by incubating pieces in 0.3% collagenase for 45 minutes and then in 0.25% Viokase for 15 min. The cells were washed, suspended ($\simeq 5 \times 10^5$ cells/ml) in medium containing 10% bovine serum after which 1 ml of suspension was transferred to each well of multiwell culture plates (Costar Inc., Cambridge, MA). Cells were grown for 4 days with medium changes at 24-hr intervals, beginning on day 2. Treatments were on day 5 in serum free medium.

Medium was a 1:1 (vol/vol) TC 199-Eagles minimal essential medium (MEM) supplemented with 10% MEM essential and 10% MEM nonessential amino acid mixtures. Medium was buffered to a final pH of 7.4 with $NaHCO_3$ (28 m*M*) and HEPES (25 m*M*). Stock solutions of thyrotropin releasing hormone (TRH) and arginine vasotocin (AVT) were in medium alone. Melatonin, serotonin (oxalate salt), 5-hydroxytryptophol and 5-methoxytryptophol were dissolved in 95% ethanol and then diluted with medium; the final concentration of ethanol in the cultures was 0.1%. Dopamine–HCL (DA) and norepinephrine–HCL (NE) were in medium containing 1% ascorbic acid to prevent their oxidation. These compounds were added to cultures in 10 μl vol, and an equal volume of vehicle was added to controls.

Experimental design. Pituitary cells were incubated for 12 hr with the compounds investigated. The concentration of each compound used is shown in Fig. 1. There were six replicates per treatment concentration, and each compound was tested in at least two separate experiments. Results for each compound were similar among replicates, therefore only results from a single experiment are presented for each. The medium was assayed for PRL as previously described (8).

Statistical analysis. The data were analyzed by one-way analysis of variance (9). Differences due to main effects were determined by orthogonal contrasts (10). Changes in PRL accumulation in the medium, with increasing concentrations of each compound tested, were analyzed by polynomial regression analysis (9).

Results. Accumulation of PRL in medium of cultures incubated for 12 hr with various compounds indigenous to the bovine pineal is in Fig. 1. TRH over the range 10^{-11}–10^{-8} *M* increased PRL 10, 26, 46, and 57% relative to controls without TRH. Release was linearly related to the log dose of TRH ($P < .005$). Addition of 10^{-7} *M* TRH did not cause a further increase in PRL release.

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[3] TC 199 and Eagles minimal essential medium from Difco Labs, Detroit, MI; Viokase and amino acids from Gibco, Grand Island NY; TRH - courtesy of Dr. R. Rippel, Abbott Labs., N. Chicago, ILL; AVT - courtesy of Dr. C. Shaar, Eli Lilly, Indianopolis, IN; other pineal compounds and collagenase from Sigma; Chicago ILL.

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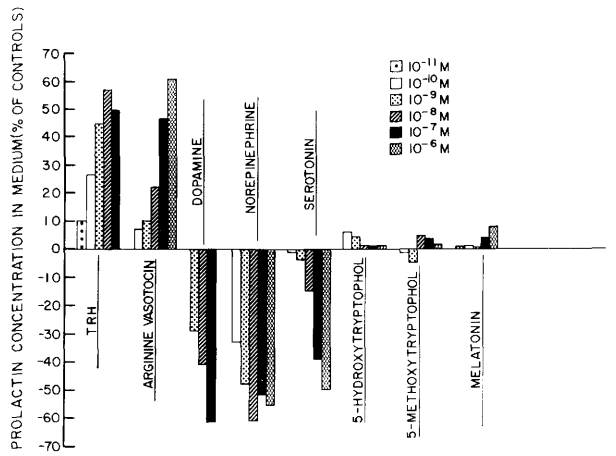

FIG. 1. PRL concentration in medium following incubation of bovine pituitary cells with varying concentrations of pineal compounds. Values are expressed as percent increase or decrease relative to controls. PRL concentrations (ng/ml) for control cultures were: TRH, 445; AVT, 444; DA, 576; NE, 214; melatonin, 275; serotonin, 268 and; 5-hydroxytryptophol and 5-methoxytryptophol, 441. Coefficients of variation for the controls ranged from 8.3 to 11.2% and were not different among groups.

Similarly, accumulation of PRL in medium increased linearly ($P < .005$) with increasing log concentration of AVT. Note however, that 10^{-8} *M* TRH elicited maximal PRL release whereas the concentration of AVT required to cause a similar increase in PRL was 100-fold greater.

In contrast to these peptides, the catecholamines, DA and NE, decreased PRL accumulation during the 12-hr incubation. The decrease in PRL due to DA was linearly ($P < .001$) related to the log concentration, over the range tested. In the case of NE, inhibition was linear through 10^{-8} *M* where inhibition appeared to reach a maximum.

Among indole compounds investigated, only serotonin affected PRL accumulation in medium. Thus, serotonin at concentrations of 10^{-10}–10^{-6} *M* decreased PRL release ($P < .001$), and the decrease was linearly related to log of the concentration used ($P < .005$). Melatonin, 5-hydroxytryptophol and 5-methoxytryptophol were without effect on PRL release from bovine pituitary cells.

Discussion. Present results demonstrate that compounds indigenous to the bovine pineal have the potential to regulate PRL secretion in this species via a direct effect on the pituitary.

TRH (11) and AVT (12) have been identified in bovine pineal glands. That TRH causes PRL release is well documented in rats (13), cattle (14), and humans (15). Arginine vasotocin has also been shown to cause release of PRL in rats (16, 17) and mice (18). Our results show that AVT, in addition to TRH, can induce PRL release via a direct effect on the bovine pituitary and that release is linearly related to log of the concentration used. However, TRH was more potent than AVT in causing PRL release in this system. Nevertheless, the possibility that AVT normally participates in the physiological regulation of PRL secretion in cattle is raised by these results. Previously, Vaughan *et al.* (16) described AVT as a "potential pineal PRL releasing factor" based on its ability to stimulate PRL release from rat pituitary explants.

Both DA and NE inhibited PRL release from bovine pituitary cells. Our results are consistent with the view that DA and NE may serve an inhibitory role in physiological control of PRL release. Others have observed direct inhibition of PRL release from rat pituitaries by DA (19, 20) and NE (19) and both decrease PRL release when infused directly into hypophyseal portal vein of rats (21). The hypothesis that these catecholamines serve as potential PRL inhibiting factors is strengthened by recent reports (22, 23) demonstrating their presence in portal blood.

Serotonin inhibited PRL release from bovine pituitary cells and in this regard bovine pituitaries act differently from those of rats.

Thus, in rats serotonin had no direct effect on PRL secretion either *in vitro* (24) or when infused directly into the pituitary (25) *in vivo*. In contrast to serotonin, melatonin had no effect on PRL release from bovine pituitary cells which confirms results obtained with rat pituitaries (25, 26). Both serotonin and melatonin increased serum PRL when injected into the third ventricle of rats (25) or into a jugular vein of steers (Goodman and Tucker, unpublished observation). Taken together, *in vivo* and *in vitro* effects of these indoleamines suggest that both may be involved in regulation of PRL secretion but at extra-pituitary loci. Perhaps the ability of serotonin to inhibit PRL secretion by a direct effect on the pituitary is overcome by its ability to stimulate PRL secretion via extra-pituitary effects. Both 5-hydroxy- and 5-methoxytryptophol are found in the bovine pineal (27). However, neither altered PRL release from bovine pituitary cells. Effects of these compounds on PRL release have not been tested in other species or in the bovine *in vivo*.

Our results demonstrate that compounds in the bovine pineal have the potential to regulate PRL release if they are delivered to the anterior pituitary. Location of the pineal gland on the dorsal aspect of the third ventricle allows its products to be secreted directly into the cerebrospinal fluid (CSF), providing a route by which pineal compounds may be transported to the pituitary (28). Melatonin is found in the CSF of calves (29) and humans (30). Once in the CSF, pineal secretions could reach the portal blood via ependymal cells lining the median eminence (31) or by bulk flow of CSF to systemic blood (32). Hence pineal compounds by virtue of their PRL releasing and release inhibiting activities have the potential to act as extra-hypothalamic regulators of PRL release.

Summary. Compounds native to the pineal were screened using a bovine pituitary cell culture system to investigate their effects on pituitary prolactin secretion. Thyrotropin releasing hormone (10^{-11}–10^{-7} *M*) and arginine vasotocin (10^{-10}–10^{-6} *M*) increased prolactin in medium and this increase was linearly related to increasing log concentrations of each peptide. In contrast, dopamine (10^{-9}–10^{-7} *M*) and norepinephrine (10^{-10}–10^{-8} *M*) decreased prolactin accumulation in medium and decrease was linearly related to increasing log concentrations of catecholamines. Serotonin (10^{-10}–10^{-6} *M*) also inhibited prolactin release and decrease was linearly related to increasing log concentrations of serotonin. Melatonin, 5-hydroxytryptophol and 5-methoxytryptophol had no direct effect on pituitary prolactin secretion. We conclude that compounds indigenous to the pineal have the potential to regulate PRL secretion from the pituitary.

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