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# Effect of Indoles, AVT, Oxytocin, and AVP on Prolactin Secretion in Rat Pituitary Clonal (2B8) Cells (40858)[1]

KUNIHIKO HANEW,[2] MASATAKA SHIINO, AND EDWARD G. RENNELS

*Department of Anatomy, The University of Texas, Health Science Center at San Antonio, San Antonio, Texas 78284*

*Abstract.* The effects of several putative pineal hormones on prolactin (PRL) secretion from clonal pituitary cells were studied *in vitro*. The pituitary cells (2B8) were derived from Rathke's pouch epithelium of normal fetal rats, and they were shown earlier to produce only prolactin when grown in Ham's F-10 medium. The substances tested were: 5-HTP (5-hydroxytryptophan), serotonin (5-HT; 5-hydroxytryptamine), NA-5-HT (*N*-acetyl serotonin), melatonin, and the nonapeptide AVT (arginine–vasotocin). For comparison with the effects of AVT, oxytocin, and AVP (arginine–vasopressin), the neurohypophyseal hormones which are analogs of AVT were also used. After 6 hr of incubation in the presence of these agents, the cells were separated and the media were collected. The media were later assayed for prolactin by radioimmunoassay.

5-HTP, 5-HT, and NA-5-HT (each $10^{-11}$ to $10^{-6}$ *M*) had no direct effect on PRL secretion from the 2B8 clonal cells. In contrast, melatonin clearly enhanced PRL secretion over the range of concentrations used. AVT also stimulated PRL secretion in low concentrations ($10^{-11}$ and $10^{-10}$ *M*), although it inhibited PRL secretion in much higher concentrations ($10^{-8}$ to $10^{-6}$ *M*). In contrast, oxytocin and AVP did not evoke any response in PRL secretion from these cells. We conclude that the pineal hormones melatonin and AVT, in physiological concentrations, may have direct stimulatory roles in PRL secretion.

It has been reported that bovine pineal extracts enhance the release of rat pituitary prolactin (PRL) *in vitro* (1). The pineal gland contains several indoleamines such as 5-HTP (5-hydroxytryptophan), serotonin (5-HT; 5-hydroxytryptamine), NA-5-HT (*N*-acetylserotonin), melatonin, and the nonapeptide AVT (arginine–vasotocin) (2, 3). These substances can possibly be released into the cerebrospinal fluid (CSF) from the pineal gland (3–6). Since there may also be a transport from the third ventricle to the hypothalamo–pituitary portal system (7), it is speculated that these pineal substances may have a physiological regulatory role in PRL secretion. In order to study whether or not putative hormones from the pineal gland may have a possible modulatory role on pituitary mammotrophs, we examined the effects of 5-HTP, serotonin, NA-5-HT, melatonin, and AVT on PRL secretion from rat pituitary clonal (2B8) cells (8) *in vitro*. As a comparison of the effect of AVT, oxytocin and AVP (arginine–vasopressin), the neurohypophyseal hormones which are analogs of AVT, were also used in this study. Preliminary results of this study were previously reported in abstract form (9).

*Materials and methods.* Pituitary clonal (2B8) cells were used in this study. These cells were cultured in 25-cm$^2$ tissue flasks (Corning, No. 25100) containing Ham's F-10 medium with serum (10% fetal calf serum and 2.5% horse serum). The cells were incubated in this medium at 37° for 3 days. The medium was then discarded and 5-HTP, serotonin, NA-5-HT, melatonin, AVT, oxytocin, or AVP[3] was added to the cells in fresh Ham's F-10 medium lacking

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[2] Postdoctoral Fellow: Dr. Hanew is on leave from the second Department of Medicine, Tohoku University School of Medicine, Sendai 980, Japan.

[3] The sources of the agents used were as follows: 5-HTP, Sigma; 5-HT (5-HT creatine sulfate), Regis Chem.; NA-5-HT, Sigma; melatonin, Sigma; AVT (AVT acetate hydrate), Bachem Inc.; oxytocin, Sigma; AVP, Sigma.



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serum. Melatonin and the other indoles were first dissolved in 5% ethanol. The solution was then diluted with Ham's F-10 medium so that the final concentration of ethanol in the culture medium was less than 0.05%. The concentrations of the seven agents studied ranged between $10^{-11}$ and $10^{-6}$ *M*. 2B8 cells cultured in serum-free Ham's F-10 medium were used as controls. Usually each flask contained about $4 \times 10^6$ cells and 12 flasks were used for control and 6 flasks used for the different concentrations of each agent. After 6 hr of incubation in the presence of these agents, the cells were separated and the media were collected. These samples were kept frozen at −27° until assayed. Rat PRL was measured in duplicate by radioimmunoassay (10). The rat PRL kit was kindly provided by NIAMDD, through the courtesy of Dr. A. F. Parlow. Intra- and interassay coefficients of variations were 2.1 and 4.8%, respectively. The data were subjected to analysis of variance followed by the Student–Newman–Keuls' test for comparing the differences between group means.

*Results.* After administration of 5-HTP into the flasks, no change in PRL secretion[4] was observed compared with the controls (Table I). Similarly, serotonin and NA-5-HT, the intermediate substance in the transformation of serotonin to melatonin, failed to evoke a significant response in PRL secretion (Table I). In contrast, melatonin clearly enhanced PRL secretion in concentrations of $10^{-11}$ to $10^{-6}$ *M* (Fig. 1). Although the responses were not linear, responses to concentrations of $10^{-9}$ to $10^{-7}$ *M* were statistically significant compared with the amount of PRL secreted into the control flasks ($P < 0.01$). The greatest response was observed at the concentration of $10^{-9}$ *M*. When AVT was added to the cultures (Fig. 2), a remarkable stimulation of PRL release was observed at low concentrations of $10^{-11}$ ($P < 0.01$) and $10^{-10}$ *M* ($P < 0.01$). Higher concentrations ($10^{-8}$ to $10^{-6}$ *M*) of AVT suppressed the secretion of PRL. However, this suppression proved to be statistically significant only at the $10^{-7}$ *M* concentration ($P < 0.05$). On the other hand, the administration of oxytocin and AVP to 2B8 cells did not produce any change in PRL release into the medium (Table I).

*Discussion.* The results of this study indicate that the pineal hormones, melatonin, and AVT may have direct modulatory roles on PRL secretion from a clonal strain (2B8) of rat mammotrophs, although other pineal indoles (5-HTP, serotonin, and NA-5-HT), and AVT analogs (oxytocin and AVP) had no direct effect on these cells. It is well known that serotonergic pathways participate in the regulation of PRL secretion (11–13). However, the exact site of serotonin action on PRL secretion has not been clearly determined. Lawson and Gala (14) demonstrated a prompt release of rat PRL after intraarterial administration of serotonin. However, Lu *et al.* (15) did not observe such an effect after intravenous administration of serotonin, although they had showed a significant elevation of PRL levels using 5-HTP. It has also been reported that serotonin injected directly into the pituitary portal vessels did not alter the plasma concentrations of rat PRL, while intraventricular administration of serotonin elevated the plasma PRL levels (16). 5-HTP, but not serotonin, can easily cross the blood brain barrier, and systemic administration of 5-HTP elevates the brain concentration of serotonin (17, 18). From these observations it was postulated that serotonin would elevate the PRL levels either via the stimulation of the PRF (PRL-releasing factor) or the inhibition of the PIF (PRL-inhibiting factor) secretion (15, 16).

Our negative *in vitro* results with serotonin are in marked contrast to the findings of Padmanabhan *et al.* (19) who examined the effects of several pineal compounds on PRL secretion from dispersed bovine pituitary cells. They reported a marked inhibitory effect of serotonin and a lack of effect

[4] The values obtained represent the prolactin released from the cells over a 6-hr incubation; this includes some hormone present in the cells at the start of the incubation as well as an unknown amount of PRL synthesized during the incubation. Consequently, we prefer using the term PRL secretion which includes both parameters of the secretory process, i.e., synthesis and release.

TABLE I. EFFECT OF INDOLES, OXYTOCIN, AND AVP ON PRL SECRETION FROM 2B8 CELLS[a]

| Agent added | Concentration ($M$) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | $10^{-11}$ | $10^{-10}$ | $10^{-9}$ | $10^{-8}$ | $10^{-7}$ | $10^{-6}$ |
| 5-HTP | 40.8 ± 0.7 | 41.8 ± 0.8 | 42.1 ± 2.2 | 40.3 ± 0.6 | 40.7 ± 0.6 | 39.1 ± 0.6 | 40.0 ± 0.8 |
| 5-HT[b] | 7.3 ± 0.5 | 7.5 ± 0.8 | 7.0 ± 0.3 | 7.0 ± 0.7 | 7.2 ± 0.6 | 7.4 ± 1.1 | 7.2 ± 0.5 |
| NA-5-HT | | 49.6 ± 3.0 | 48.1 ± 2.5 | 48.4 ± 2.6 | 51.2 ± 3.9 | 52.6 ± 4.3 | 51.0 ± 0.9 |
| Oxytocin | 48.5 ± 2.7[c] | 53.1 ± 5.5 | 51.2 ± 1.9 | 50.4 ± 4.0 | 44.5 ± 1.5 | 44.7 ± 0.9 | 45.0 ± 2.5 |
| AVP | | 45.8 ± 3.0 | 48.0 ± 2.3 | 49.3 ± 2.4 | 48.4 ± 2.5 | 50.0 ± 4.2 | 56.6 ± 3.3 |

[a] Cells were incubated at room temperature for 6 hr prior to collection of media for RIA of PRL. All values are mean ± SEM (ng/ml) indicating equivalent NIAMDD-Rat-PRL-RP-1 in the conditioned medium.
[b] Number of cells ($0.5 \times 10^6$/flask) employed was less than in all other experiments.
[c] Same controls (25 samples) were used for experiments of NA-5-HT, oxytocin, and AVP.

with melatonin. These differences may be due to the fact that these workers were using a mixed population of bovine pituitary cells rather than a clonal strain. In addition, damage to cell surface receptors may also be a factor in studies utilizing dispersed pituitary cells.

Kamberi *et al.* (16) showed a stimulation of PRL release in rat plasma after intraventricular administration of melatonin. However, they observed no response in PRL secretion when melatonin was administered directly into pituitary portal vessels. Therefore, it was speculated that melatonin may reduce the release of PIF or enhance the release of PRF (16). In the present study, we observed a stimulation of PRL release from 2B8 cells using melatonin with the concentrations ranging from $10^{-11}$ to $10^{-9}$ $M$, but this effect of melatonin was attenuated at the higher concentrations of $10^{-8}$ to $10^{-6}$ $M$. Such nonlinear responses have been reported using estradiol, dopamine (20), TRH (21), and LH-RH (22). The concentration of melatonin used by Kamberi *et al.* (16) was extremely high ($8.6 \times 10^{-3}$ $M$/min for 30 min). Therefore, it is not surprising that such an extremely high dose did not evoke a PRL response. In the rat, the physiological concentration of plasma melatonin is reported at 10 to 45 pg/ml ($4.3 \times 10^{-11}$ to $1.9 \times 10^{-10}$ $M$) (23). We observed increasing stimulatory effects of melatonin at the concentrations of $10^{-11}$ to $10^{-9}$ $M$ which are close to the peripheral plasma concentration of melatonin. In general, the concentration of melatonin in cerebral spinal fluid (CSF) is similar to that of the plasma in several species (24, 25), and it is thought that melatonin in the CSF can reach the pituitary gland via a special link between the third ventricle and the pituitary gland

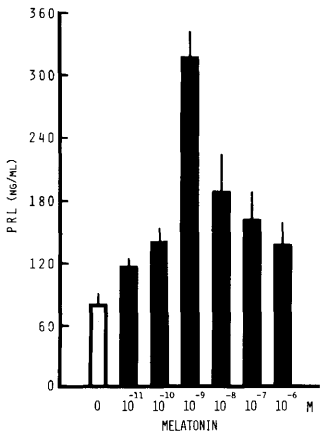


FIG. 1. Effect of melatonin administration on PRL secretion (mean ± SEM) into culture medium from clonal (2B8) cells.

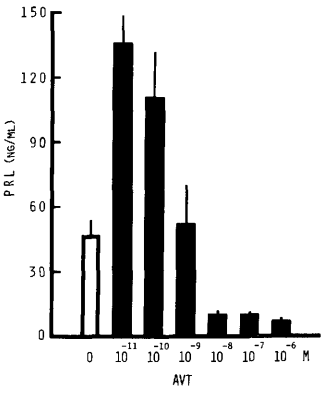


FIG. 2. Effect of arginine–vasotocin (AVT) administration on PRL secretion (mean ± SEM) into culture medium from clonal (2B8) cells.

(7). Hence, it is suggested that $10^{-11}$ to $10^{-9}$ *M* concentrations of melatonin are approximately the physiological concentrations reaching the pituitary gland, and that these concentrations of melatonin exert a direct stimulatory effect on PRL secretion from rat mammotrophs *in vitro*. The precursor of melatonin, NA-5-HT, had no direct stimulatory effect on PRL secretion. NA-5-HT is produced from serotonin through enzymatic conversion by *N*-acetyltransferase (26). In addition, NA-5-HT is *o*-methylated by HIOMT (hydroxyindole-*o*-methyl transferase) to form the specific pineal compound melatonin (26). Therefore, it is speculated that the *o*-methylation of NA-5-HT may have an important conformational significance on the affinity of melatonin for mammotrophs and on the stimulation of PRL secretion.

Concerning the effect of AVT on PRL secretion, Vaughan *et al.* (27) noted a dose–response effect on PRL release when slightly higher concentrations ($5 \times 10^{-9}$ to $5 \times 10^{-6}$ *M*) of AVT were added to the incubation medium containing hemipituitary glands of intact male rats. They did not observe any effect on PRL secretion when they used hemipituitaries from castrated, estrogen–progesterone (EP)-primed female rats (27), but they reported a dose-dependent PRL releasing activity of AVT in either intact or pinealectomized EP-treated male rats *in vivo* (28). On the other hand, Pavel *et al.* (29) found an inhibition of the postpinealectomy increase in PRL content in the mouse pituitary gland after injection of an extremely low concentration of AVT ($10^{-13}$ *M*) into the third ventricle. Therefore, the action of AVT on PRL secretion seems to be highly complex and AVT appears to have a wide range of actions.

In this study, AVT clearly enhanced the release of PRL from 2B8 cells at concentrations of $10^{-11}$ to $10^{-10}$ *M*. Since the peripheral plasma concentration of AVT in rats is reported at about 5 pg/ml ($4.7 \times 10^{-12}$ *M*) (2), and since AVT is probably released into CSF of the third ventricle (3), the AVT concentration in CSF would presumably be much higher than the peripheral concentrations. Therefore, $10^{-11}$ and $10^{-10}$ *M* of AVT might be physiological concentrations in the CSF, and these concentrations of AVT could directly stimulate the PRL release from normal rat pituitary glands. On the other hand, $10^{-9}$ *M* AVT had no effect on the PRL secretion from 2B8 cells, and the much higher concentrations of AVT ($10^{-8}$ to $10^{-6}$ *M*) inhibited the PRL release. It is reported that AVT is the most active biological substance known in vertebrates (30). For example, an unbelievably small amount of AVT ($10^{-7}$ pg = 60 molecules) injected into the third ventricle produced a detectable effect on plasma cortisol, possibly via the endogenous CRF (corticotropin-releasing factor), in cats (31). Since the rat pineal gland contains only 200 pg of AVT (32), $10^{-9}$ to $10^{-6}$ *M* (5245 pg/flask to 5,245,000 pg/flask) of AVT might be pharmacological doses. Therefore, we suspect that the responses of mammotrophs to those higher concentrations of AVT may have no physiological significance.

The administration of the AVT analogs, oxytocin and AVP, to 2B8 cells did not show any effect on PRL secretion. Previously, it was speculated that oxytocin has a stimulatory action on PRL secretion in rats (33). Later, however, several authors (34–36) denied such a stimulatory effect of oxytocin. Concerning the *in vitro* effects of AVP, Talwalker *et al.* (34) and MacLeod and Lehmeyer (37) observed no effect on PRL secretion in rats. From our *in vitro* experiments, it would seem that oxytocin and AVP have no direct stimulatory action on mammotrophs.

In conclusion, melatonin and AVT appear to have a physiological stimulatory effect on PRL secretion *in vitro*, although other pineal indoles (5-HTP, 5-HT, and NA-5-HT) and the AVT analogs (oxytocin and AVP) had no direct effect on PRL secretion.

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