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Changes in Vasopressin-Converting Aminopeptidase Activity in the Rat Pineal Gland During Summer: Relationship to Vasopressin Contents

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LIU, B AND J P H BURBACH *Changes in vasopressin-converting aminopeptidase activity in the rat pineal gland during summer Relationship to vasopressin contents* PEPTIDES 9(6) 1235–1240, 1988 —Vasopressin (VP)-converting aminopeptidase (VP-AP) activity and VP contents were measured in single rat pineal glands during the summer of two successive years The peptidase activity decreased significantly in August The lowest activity (±SEM) of 0 18±0 02 pmol hour^{-1} was recorded on August 14, compared to the basal activity of 0 25±0 01 pmol·hour^{-1} in July and September of 1986 The change with similar percentage occurred in the same period of 1987 The specific activity of the enzyme in the crude homogenate, 15,000 g pellet and supernatant fraction of rat pineal glands, exhibited the same pattern of variations The decrease in peptidase activity coincided with the previously reported dramatic rise in pineal VP content in early August which was confirmed in this series of experiments Another peptidase, the so-called γ-endorphin generating endopeptidase (γ-EGE) activity, and β-endorphin-related peptides in the pineal gland did not change in this period The results show that the variations of pineal VP contents and VP-AP activity during summer are not general for other peptides and peptidases The coincidence of opposite changes in VP content and VP-AP activity of the pineal gland may indicate a role of the peptidase activity to regulate the VP content

Vasopressin-converting aminopeptidase activity Pineal gland Summer Vasopressin content

VASOPRESSIN (VP) has biological functions in the periphery and the central nervous system (9,11). In contrast to the periphery, central actions of VP are also exerted by fragments of VP (6, 9, 11). An aminopeptidase activity with restricted specificity has been implicated in the formation of such active fragments (4,5). The functional role of this "VP-converting aminopeptidase (VP-AP)" activity in the metabolism of VP, however, remains to be clarified. Both VP and VP-AP activity have been found in the rat pineal gland (7,16), an endocrine organ which is important in regulation of biological rhythms (1) Recent experiments in our laboratory indicated that a diurnal rhythm of VP-AP activity exists in the rat pineal gland which is inversely related to the pineal VP contents (15). The marked rise of pineal VP contents during summer (14) provides another physiological condition for evaluation of their relationship in the pineal gland. Therefore, VP-AP activity and VP contents were measured in the rat pineal gland during the period of summer in two successive years. The specificity of the changes was investigated by comparison to another peptide system and its converting enzyme, i.e., β-endorphin-related peptides and the so-called "γ-endorphin-generating endopeptidase (γ-EGE)," a Leu-Phe cleaving endopeptidase which converts β-endorphin into γ-endorphin (12,13) The results indicate that VP-AP in the rat pineal gland may participate in the regulation of pineal VP contents.

METHOD

Materials

Radioactive materials [^{3}H-Phe^{3}]VP, ^{3}H-Phe and [^{14}C]formaldehyde were purchased from New England Nuclear Company (Boston, MA) with the specific activity of 40 0 Ci/mmol, 71 0 Ci/mmol and 53.0 mCi/mmol, respectively. Polystyrene beads (Amberlite XAD2) were from BDH chemicals (Poole, Dorset, UK). Synthetic peptides were prepared and provided by Dr. H. M. Greven and Dr. J W van Nispen (Organon International B.V , Oss, NL).

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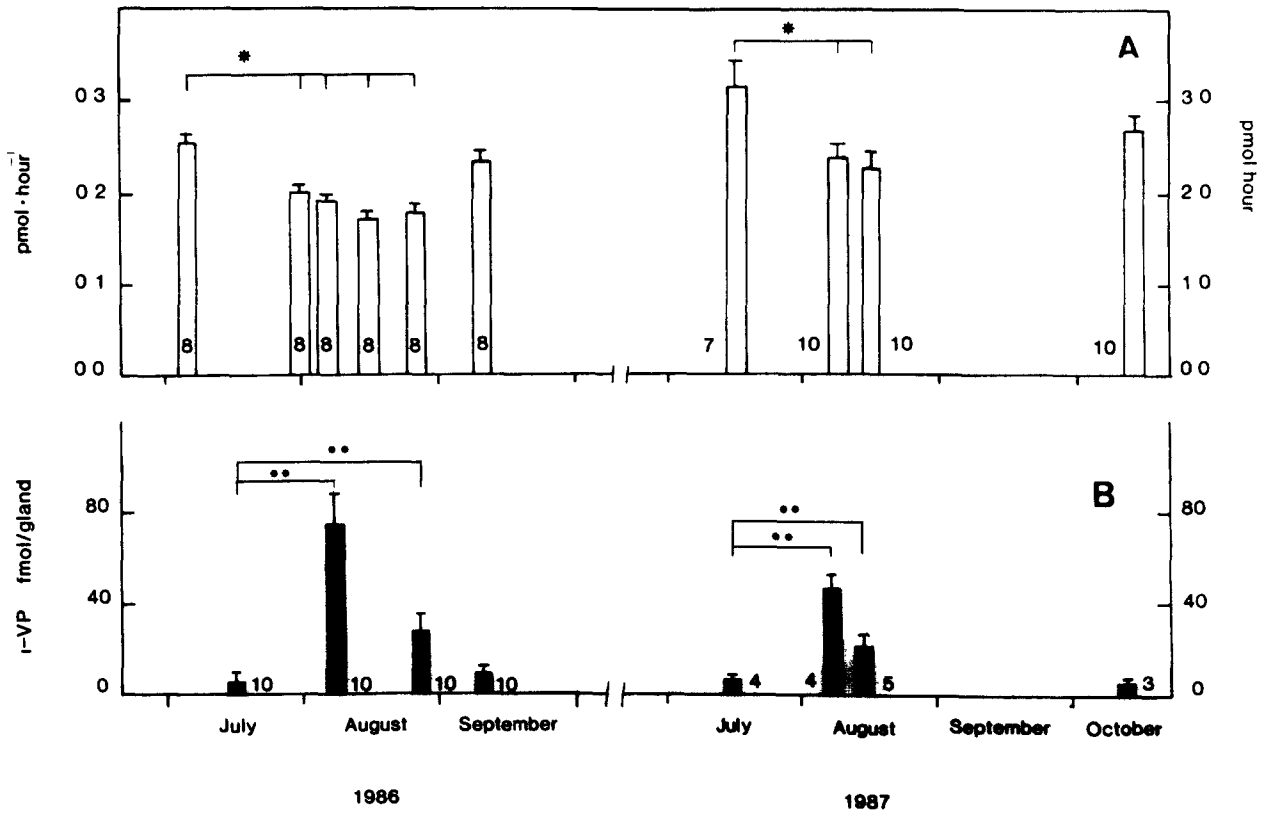

FIG 1 VP-AP activity and VP levels in the rat pineal gland during the period of summer Pineal glands were collected on July 3, July 30, August 6, August 14, August 26, September 10 of 1986 and on July 13, August 6, August 14, October 16 of 1987 VP-AP activities and VP contents were determined in individual glands (A) Total VP-AP activity per gland in the pellet fraction (1986) and homogenate (1987) (B) VP content per gland. The peptide contents were not corrected for the recovery. Values are means±SEM. The number of animals is indicated in the bar Statistical significance (*$p<0$ 05) was performed by analysis of variance (ANOVA) and the Newman-Keuls test

[^{125}I]NaI was obtained from the Radiochemical Centre, Amersham, UK. Bovine serum albumin (BSA) and human serum albumin (HSA) were from Sigma (St. Louis, MO). Dextran T-70 was from Pharmacia Fine Chemicals (Uppsala, Sweden) and charcoal from Merck (Darmstadt, FRG). All other chemical reagents were reagent grade.

Animals

Male Wistar rats (140–170 g) were housed five per cage with food and water available ad lib under standard light (light on from 0600–2000 hr) and temperature (23°C) conditions. Animals were gently handled for one week before sacrifice. Groups of rats were decapitated between 0900–1100 hr in July, August and September of 1986 and 1987. The pineal glands were quickly removed and frozen on dry ice within one minute after the decapitation and stored at −80°C.

Assay of Peptidase Activities

For the measurement of peptidase activities, single rat pineal glands were homogenized in 50 μl of 20 mM Tris-HCl, pH 7.0 by a teflon-glass homogenizer. Another 50 μl of the buffer used to wash the homogenizer was combined. Parts of the homogenate were centrifuged at 15000×g for 30 min to obtain a membrane pellet and a supernatant. Pellets were resuspended in 50 μl of 20 mM Tris-HCl, pH 7 0, by suction through a 100 μl syringe with a 16 gauge needle All procedures were performed at 0°C. Protein concentration of each fraction was determined by the method of Bradford (3).

VP-AP activity was determined in the homogenate, 15,000 g pellet and supernatant of single pineal glands (incubation concentration from 0.05 to 0.5 mg protein/ml) as described previously (4,5). In this assay, 40 μl of the enzyme preparation was incubated with 10 μl (10^4 dpm) of [^{3}H-Phe^3]VP in 20 mM Tris-HCl, pH 7.0, at 37°C for 30 min. To determine the γ-EGE activity 10 μl of the homogenates of single pineal glands (incubation concentration from 0.1 to 0.3 mg protein/ml) were incubated with 10^4 dpm of a ^{14}C-labelled pentapeptide Ac-Val-Thr-Leu-Phe-[^{14}C]$(CH_3)_2$Lys-NHCH$_3$ as the substrate in a total volume of 50 μl of 20 mM Tris-HCl, pH 8.5, at 37°C for 30 min as described before (12). Both assays were performed under conditions of linearity between enzyme concentration and substrate conversion. The enzymatic reactions were stopped by adding 50 μl acetic acid to a concentration of 1 M and boiling for 5 min. Membranes were removed by centrifugation at 10,000×g for 10 min Separations between substrate and product were performed on polystyrene minicolumns (Amberlite XAD-2) by stepwise elution with ethanol-water mixtures as described elsewhere (5,12).

TABLE 1

SPECIFIC ACTIVITY OF VP-CONVERTING AMINOPEPTIDASE (pmol hour^{-1}·mg protein^{-1}) IN THE HOMOGENATE, 15,000 g PELLET AND SUPERNATANT FRACTIONS OF THE RAT PINEAL GLAND DURING THE SUMMER OF 1987

	July 15	August 7	August 14	October 12
Homogenate	2 33 ± 0 14	1 75 ± 0 12*	1 70 ± 0 12*	2 18 ± 0 21
Pellet	4 15 ± 0 34	3 39 ± 0 29	3 29 ± 0 42	4 13 ± 0 49
Supernatant	2 60 ± 0 34	2 34 ± 0 33	1 90 ± 0 30	2 33 ± 0 39

Each group consisted of 10 animals *$p<0$ 05 analyzed by ANOVA and the Newman-Keuls test

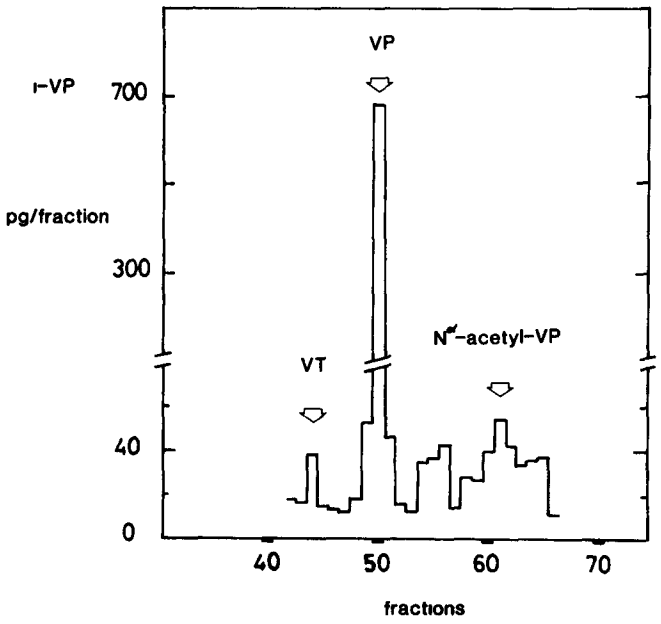

FIG 2 HPLC profile of VP-immunoreactivity of the rat pineal gland Fifty rat pineal glands were collected in August Glands were extracted and analyzed by HPLC The gradient run linearly from 0 to 90% of solvent B in 60 min at a flow rate of 2 ml/min Fractions of 1 ml were collected in polystyrene tubes containing 100 μl of 0 1% BSA VP-immunoreactivity of each fraction was determined by VP-RIA Before fraction 40 the VP-immunoreactivity was at the baseline level Values were not corrected by the recovery The elutions of marker peptides are indicated by arrows VT vasotocin

Tissue Extraction and Radioimmunoassays (RIA)

Single rat pineal glands were extracted at 100°C in 1 N acetic acid (50 μl/gland) for 10 min, then kept at 0°C for 10 min and disrupted by sonication (30 sec/gland) The homogenates were centrifuged at 12,000×g at 4°C for 30 min and the supernatants were dried in RIA tubes in vacuo at 40°C in a Speed Vac Concentrator Dried samples were then dissolved in RIA buffers and assayed.

For the measurement of VP-immunoreactivity, antibody W1E, specific for the C-terminus of VP was used (17). The procedure of the RIA was performed as described before (5,17). The sensitivity of the assay was 0.5 fmol/tube (at 10% tracer displacement) and tracer displacement of 50% occurred at 8 fmol. Half of the pineal extracts was used for the measurement Intraassay variability was about 10%. Interassay variability was about 20%.

β-Endorphin immunoreactivity was measured with antiserum B4. The sensitivity of the assay was 2.0 fmol/tube Both inter- and intraassay variabilities were about 10% Tracer displacement of 50% occurred at 60 pg. The assay procedure and antibody specificity has been described before (2).

Dilutions curves of the pineal extracts were parallel to the standard curves in both RIAs. All the data were processed on an IBM personal computer and analyzed by ANOVA and the Newman-Keuls test.

High-Performance Liquid Chromatography (HPLC)

VP-immunoreactivity and β-endorphin-immunoreactivity of the rat pineal gland were analyzed by HPLC on a μBondapak C18 column using a methanol-ammonium acetate solvent system (17). Solvent A was made of 10 mM ammonium acetate, pH 4.15, solvent B was methanol containing 0.15% of acetic acid (v/v) Linear gradients were employed for separations as indicated in the legends. The flow rate was 2 ml/min and fractions of 1 ml were collected. Peptide immunoreactivities were determined in each fraction by RIAs

RESULTS

Measurements of pineal VP-AP activity were conducted in the pellet fractions of the glands collected in 1986, and in pellet, supernatant fractions and homogenates of the glands collected in 1987 The enzyme activity of July 1986 decreased significantly at the beginning of August, reaching its lowest level on August 14, and then gradually increased to initial levels in September (Fig. 1A). No significant difference of the activity was observed between the groups of July and September. The average value (±SEM) of total pellet-associated activity per gland of the groups of July 3 and September 10 was 0.25±0.01 pmol·hour^{-1} (n=16) The value recorded on August 14 was 0.18±0.02 pmol hour^{-1} (n=8). The enzyme activity in all groups of August was significantly lower than that in July

This observation was reproduced in the summer of 1987. Total VP-AP activity in the homogenates of the pineal glands showed a similar variation. The enzyme activity was reduced significantly by 28% on August 14 as compared to the value of July (Fig 1A) These changes also occurred for the specific activity of the enzyme in the homogenates, pellet and supernatant fractions (Table 1). The specific activities of VP-AP were reduced by 25%, 21% and 23%, respectively, on

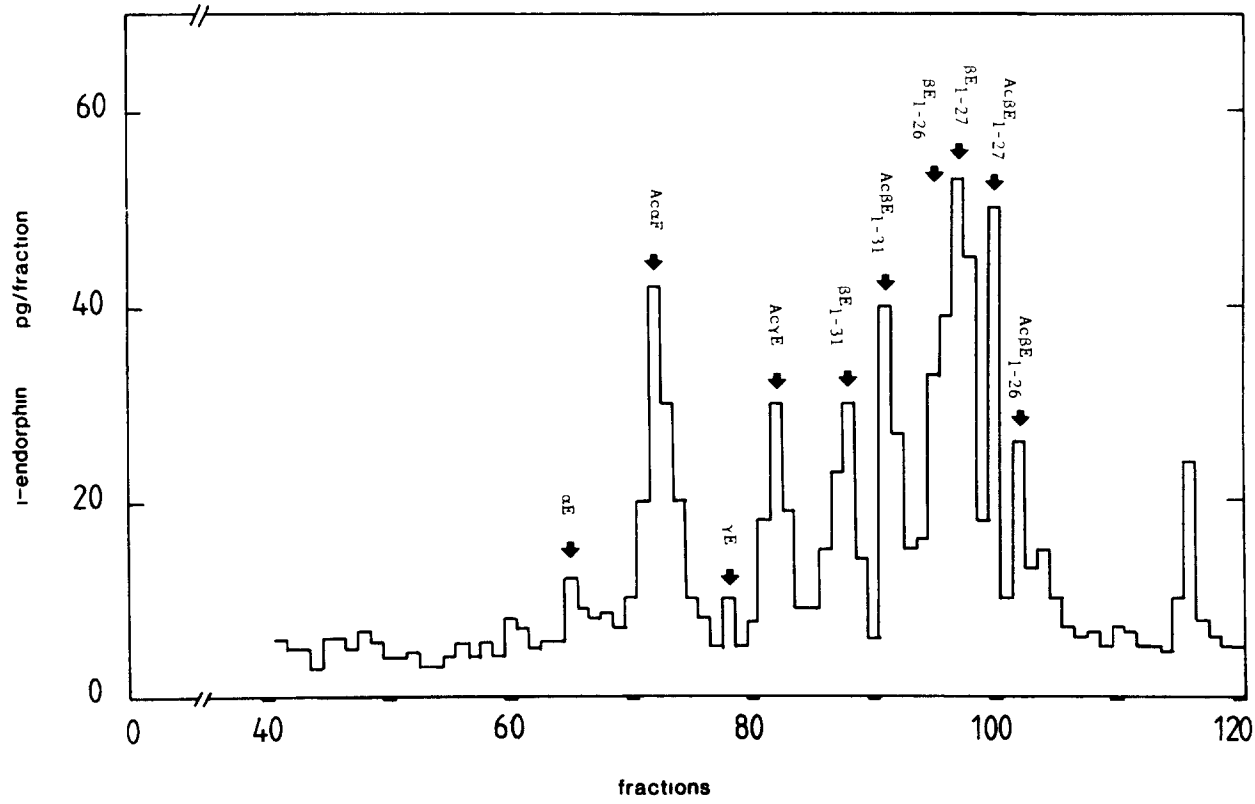

FIG 3 HPLC profile of the β-endorphin immunoreactive substances of the rat pineal gland Fifty glands collected in July of 1987 were extracted and analyzed by HPLC The gradient run linearly from 0 to 90% of solvent B in 60 min at a flow rate of 2 ml/min. The elution positions of marker peptides are indicated by the arrows Fractions of 1 ml were collected in polystyrene tubes containing 100 μl of 0 1% BSA Parts of the fractions were assayed for β-endorphin immunoreactivity Data were not corrected by the recovery

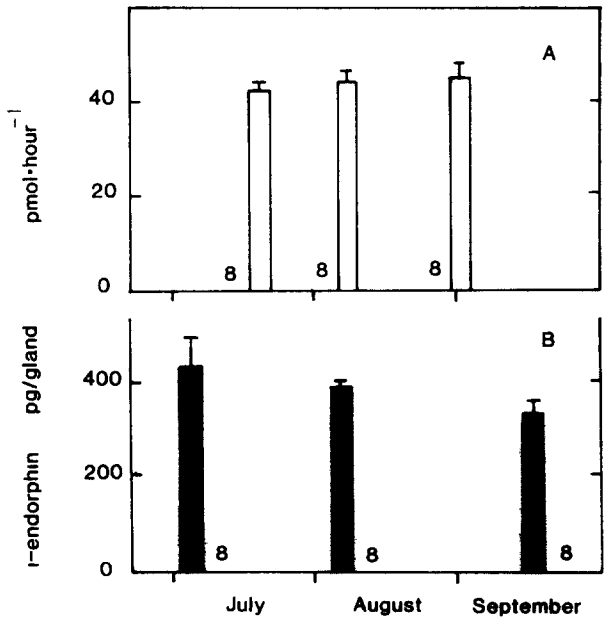

FIG 4 γ-EGE activity and β-endorphin immunoreactivity in single rat pineal glands during the period of summer Pineal glands were collected on July 3, July 13, August 6, September 1 and September 16 of 1986 (A) Total γ-EGE activity per gland (B) Content of β-endorphin immunoreactivity per gland Values are shown as mean±SEM The number of animals is indicated in the bars

August 14 as compared to the average value of July and October Statistical significance was only found for the homogenate. The protein contents in these different enzyme preparations, however, did not show significant variation during this period.

VP contents of the pineal gland were concomitantly measured in the same period. As reported before (14), the pineal VP contents rose rapidly at the beginning of August and decreased again within the same month. All values for VP obtained in August of 1986 were significantly higher than those of the groups of July and September. This observation was confirmed in 1987 (Fig. 1B). The value of 47.6±6.7 pg/gland (n=4) measured on August 6 was significantly higher than the average value of 6.3±2.0 pg/gland (n=7) measured in July and October. HPLC analysis of VP-immunoreactivity in August showed that the major peptide was VP (Fig. 2). In addition, about 10% of N^{α}-acetyl-VP in the total content of VP-immunoreactivity and a small amount of vasotocin-coeluting substance (17) was detected. The highest content was measured on August 6 of the two successive years.

For comparative purpose pineal levels of β-endorphin-immunoreactivity and γ-EGE activity were also measured. HPLC analysis of this immunoreactivity showed the presence of α-, β- and γ-endorphin and their N^{α}-acetylated forms. Major forms resembled in HPLC the acetylated and nonacetylated forms of β-endorphin-(1–27) (Fig. 3). Total contents of pineal β-endorphin immunoreactivity did not change in the period between July and October

(Fig. 4B). γ-EGE activity in rat pineal glands did not show significant variations during the period of summer (Fig. 4A). Measurement of γ-EGE activity resulted in similar values with the samples collected on July 17, August 6 and September 1 of 1986

DISCUSSION

The data show that variations of VP-AP activity and VP contents in rat pineal glands occur during summer they coincide in August and are in opposite directions. In August of two successive years, the peptidase activity decreased significantly, while VP levels dramatically increased No change occurred in another peptide system, i.e, β-endorphin and γ-EGE activity in this period

Changes in pineal VP-AP activity occurred in both 15,000 g pellet and supernatant fractions of the pineal glands Previously we have observed that VP-AP activity of brain tissue exists in a membrane-associated and soluble form and that the two forms have very similar properties (Burbach and Tan, unpublished). For these reasons we have also measured VP-AP activity in homogenates of the tissue collected in 1987 The results indicated that VP-AP activity is generally lower in all fractions of the pineal gland in August. Whether this decrease is caused by a decrease in the total amount of the enzyme or Vmax, or an increase of Km value remains to be investigated

VP-AP activity has been discovered in in vitro studies on the metabolism of VP. It appeared that the main VP metabolites were products of an aminopeptidase action on VP (4). Several of these VP metabolites displayed biological activities in the central nervous system with higher potency than VP (6,10). The involvement of VP-AP in the metabolism of endogenous VP in vivo has been indicated by the presence of similar VP metabolites in brain extracts (8,20) VP-AP activity is widely distributed in rat brain, including the pineal gland (7). In previous experiments we observed that VP-AP activity in the pineal gland displayed a diurnal variation with a rapid, short-lasting rise of enzyme activity at the onset of light. Simultaneously with this rise the content of VP decreased (15). An inverse change of peptide and peptidase has also been described for the diurnal change in pineal levels of angiotensin II and angiotensin converting enzyme (19). The present study suggests further a possible causal relationship between the VP-AP activity and VP contents in the rat pineal gland A marked rise of pineal VP contents which occur in early August has been reported before (14) In August of two successive years the dramatic rise of VP was accompanied by a significant decrease of VP-AP activity Although the VP-AP activity may have a role to regulate the pineal VP content, it seems likely that other factors, such as biosynthesis, release and CSF level of VP, also contribute to the summer rise of pineal VP These factors will be investigated.

Further arguments for a possible role of VP-AP activity in the control of VP contents may come from measurement of VP metabolites in the pineal gland However, the presently available methods lack as yet the required sensitivity to carry out such experiments, since VP metabolites have low crossreactions with our VP antiserum W1E (17)

The summer rise of pineal VP was confirmed here. HPLC analysis showed that the rise in immunoreactivity was mainly due to VP but not N^{α}-acetyl-VP recently discovered (16) or a vasotocin-coeluting substance (17) Neither the rise in pineal VP contents nor the decrease in VP-AP activity represents a general phenomenon for peptides and peptidase in the pineal gland. No change was observed in the β-endorphin system of the gland The total β-endorphin-immunoreactivity as well as the γ-EGE activity remained the same during the period of summer Furthermore, HPLC analysis showed a variety of β-endorphin-related peptides and a high degree of acetylation to be present in the pineal gland.

Variations of the VP-AP activity and VP contents occurred in animals exposed to an environment of constant temperature, humidity and photoperiod It has been speculated that air-born factors may be involved in this annual rhythm (18) The biological significance of the seasonal variations in the pineal VP system may be related to modulation of pineal functions.

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