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PINEAL PROTEIN SYNTHESIS HIGHLY SENSITIVE TO ACTH-LIKE NEUROPEPTIDES

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SUMMARY

Pineal protein synthesis was studied in vitro over a period of 6–8 h after dissection. The level of protein synthetic activity of the pineal gland was greatly dependent on the time of dissection showing a maximum at midnight and a minimum at 10.00 h, 2 h after onset of light. Low concentrations of $ACTH_{1-24}$ (down to 10^{-11} M) could stimulate protein synthesis in vitro. The sensitivity to hormonal stimulation showed a circadian variation similar to that observed in the basal protein synthetic activity. Furthermore, overall synthetic activity appeared to be under neural influence. These neural and hormonal influences seemed to be mediated by β-receptor stimulation and cyclic AMP. Structure-activity studies of the ACTH-effect on pineal protein synthesis gave results similar to those previously observed for excessive grooming behaviour, synaptic plasma membrane phosphorylation, adenylcyclase-activity and cell-free protein synthesis in brain.

It was concluded, that overall pineal protein synthesis is both under neural and hormonal control. The action of ACTH on protein synthesis rate might be mediated by a calcium-dependent release of norepinephrine followed postsynaptically by β-receptor activation, cAMP production, and stimulation of translation.

INTRODUCTION

Pituitary neuropeptides appear to be involved in learning and memory processes and to exert direct effects on central nervous structures[4]. In the case of ACTH and its congeners, their central mechanism of action involves changes in neurotransmitter turnover[30], in RNA and protein synthesis[5,10,22,26], in cyclic nucleotide levels[32] and in

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phosphorylation of membrane proteins and lipids[15,35,36]. The observed changes in phosphorylation of the synaptic membranes might be related to neurotransmitter action[13]. The present study focusses on neuropeptide-induced changes in protein synthesis in relation to synaptic events.

The pineal gland has been mentioned by others as an easy-to-manipulate model system for the study of the neuroendocrine mechanisms[2]. For a major part, pineal enzyme activities seem to be under (nor)adrenergic control[16,19,25,33]. Because of its small size, regulation of metabolic processes in the pineal can be easily studied by incubation of the whole gland together with adhering presynaptic terminals in vitro under defined conditions. A further advantage is, that its protein synthesis rate in vitro can be maintained at a level comparable to that in vivo[7].

The present data show that the overall in vitro rate of pineal protein synthesis is under control of noradrenergic mechanisms and is highly sensitive to ACTH (corticotrophin) and cyclic AMP. In addition, a profound circadian rhythm was observed both in basal synthetic activity and in sensitivity to neuropeptides (Fig. 7).

MATERIALS AND METHODS

Animals and surgery

Female Wistar rats of an inbred strain (weighing 120–130 g) were housed 6 per cage and maintained at 26 ± 2 °C and a relative humidity of 60 %, on a 12 h light:12 h dark cycle with lights on from 08.00 to 20.00 h. Food and water were available ad libitum. When not indicated otherwise, the animals were sacrificed by decapitation at 10.00 h. The skull was lifted and the pineal was dissected free from the skull. The whole procedure took less than 2 min. In case the animals were sacrificed during the dark period, the procedure was carried out using red light.

Hypophysectomy was performed via the transauricular route 3 weeks prior to the experimental day and the effectiveness of the operation was checked by determination of plasma corticosteroid levels in the decapitation blood. The operation was considered successful when the level was less than 5 μg/ml. Bilateral decentralization of the pineal gland was performed under Hypnorm anesthesia. Ptosis was used to monitor the success of the operation. Animals were used 5 days after the surgery.

For both surgeries, sham operations were performed, leaving the pituitary and the nerves intact, respectively.

Valine-incorporation into pineal protein in vitro

The measurement of protein synthesis rate in vitro was carried out essentially according to Dunlop et al.[6,7]. After dissection, each pineal was preincubated in 0.5 ml of the incubation medium in a 4 ml plastic tube under pure oxygen and gyrotory shaking at 37 °C for 2 h. The medium consisted of HEPES (N2-hydroxyethylpiperazine-N^1-2-ethane sulfonicacid) buffer pH 7.6, 25 mM; NaCl, 118 mM; KCl, 4.4 mM; $MgSO_4 \cdot 7H_2O$, 1.3 mM; K_2HPO_4, 1.5 mM; glucose, 12 mM, reflecting ion conditions of cerebrospinal fluid[6] except that Ca^{2+}-ions were omitted. Incubation was started by transferring the pineal to a tube with fresh medium containing 1 mM [1-

^{14}C]valine (spec. act. 0.5 μCi/μmol, Amersham) and the peptide and/or drug to be tested. Incubation was performed under oxygen at 37 °C and was stopped by transferring the pineal to an ice-cold solution of 0.25 M perchloric acid (PCA) containing 1 mM [^{12}C]valine. Subsequently, the pineals were washed individually by careful sequential passing through the following solutions: ice-cold 0.25 M PCA containing 1 mM valine for 16 h to extract low molecular compounds; 0.5 M PCA at 90 °C for 20 min followed by an ice-cold, 0.25 M PCA wash to extract nucleic acids; methanol, methanol:chloroform (1:1) and ether to extract lipids. Next, the pineal residues were thoroughly dried at 40 °C. The protein content was determined by weighing the residues and occasionally checked by the method of Lowry et al.[17]. Subsequently, the residue was dissolved in a tissue solubilizer (Soluene 350, Packard) and the solution was mixed with a scintillation cocktail containing xylene (Lipoluma, Lumac). A small volume of water was added to avoid chemoluminescence. ^{14}C-radioactivity was measured in a liquid scintillation counter (Berthhold, BF8000) at 88% efficiency. By amino acid analysis, it was established, that the radioactivity incorporated into proteins was at least 95% in valine[23]. The incorporation was expressed as dpm/mg protein. This procedure resulted in a very low experimental variation and allowed determination of valine-incorporation into amounts of proteins as low as 30 μg.

RESULTS

Time-curve of labeling of pineal proteins in vitro and of the effect of ACTH

The initial kinetics of the incorporation of [1-^{14}C]valine into pineal proteins in vitro are shown in Fig. 1A. It would seem, that the incorporation rate levelled off within the first two hours of incubation (Fig. 1A). However, when the incorporation

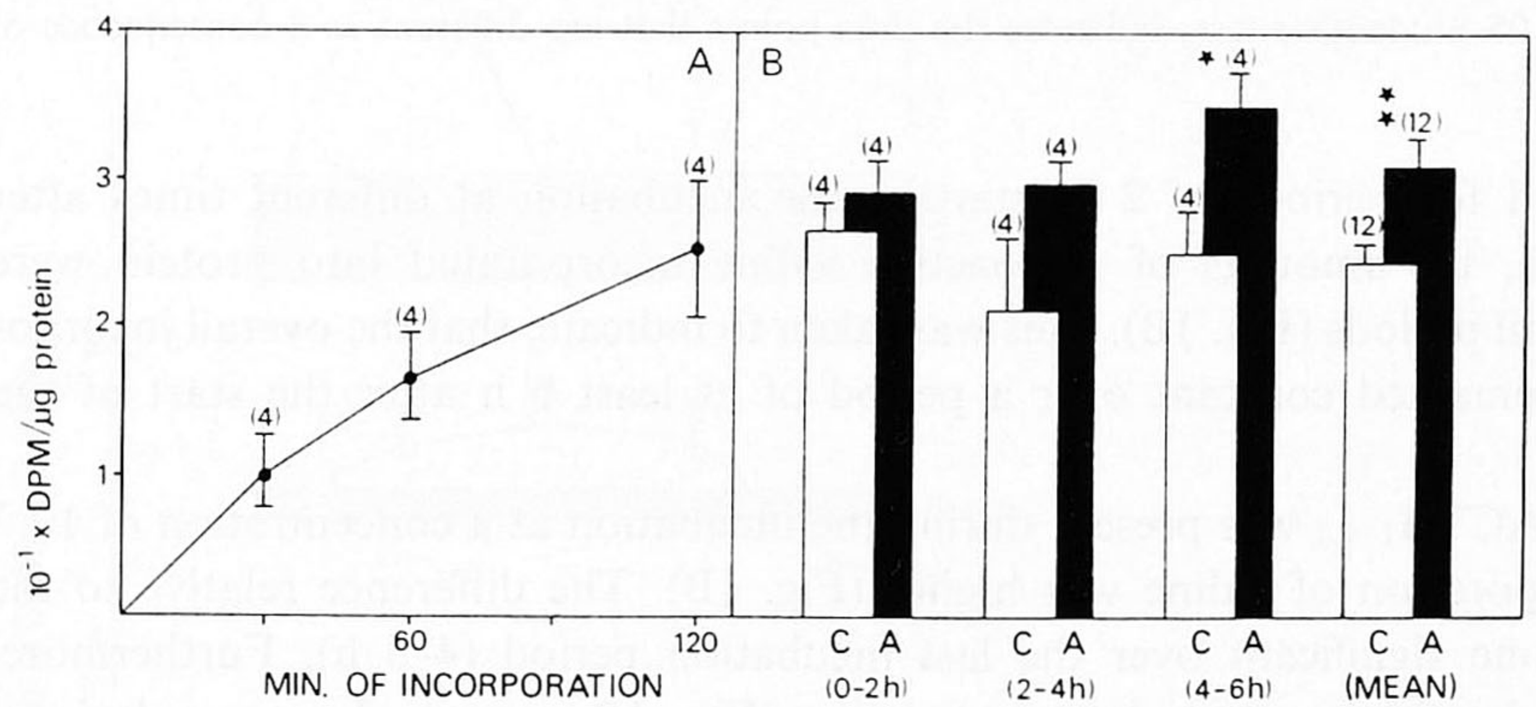

Fig. 1. Time curves of the incorporation of valine into pineal proteins in vitro (A) and of the effect of $ACTH_{1-24}$ (B). The pineals were dissected at 10.00 h. A: pineals were preincubated for 2 h and, subsequently, incubated with 1 mM [1-^{14}C]valine for the times indicated. B: the incorporation of [1-^{14}C]valine was measured over periods of 2 h after a preincubation time of 2 h (0–2 h), 4 h (2–4 h) or 6 h (4–6 h), respectively. Bars represent mean ± S.E.M.; the numbers of animals are indicated in brackets. $ACTH_{1-24}$ (10^{-7} M) was added at time 0 of the incubation (black bars A). Controls were incubated under similar conditions but without ACTH (open bars, C). *$2P < 0.05$ Student's t-test. **$P < 0.01$ 2-way analysis of variance, A vs C.

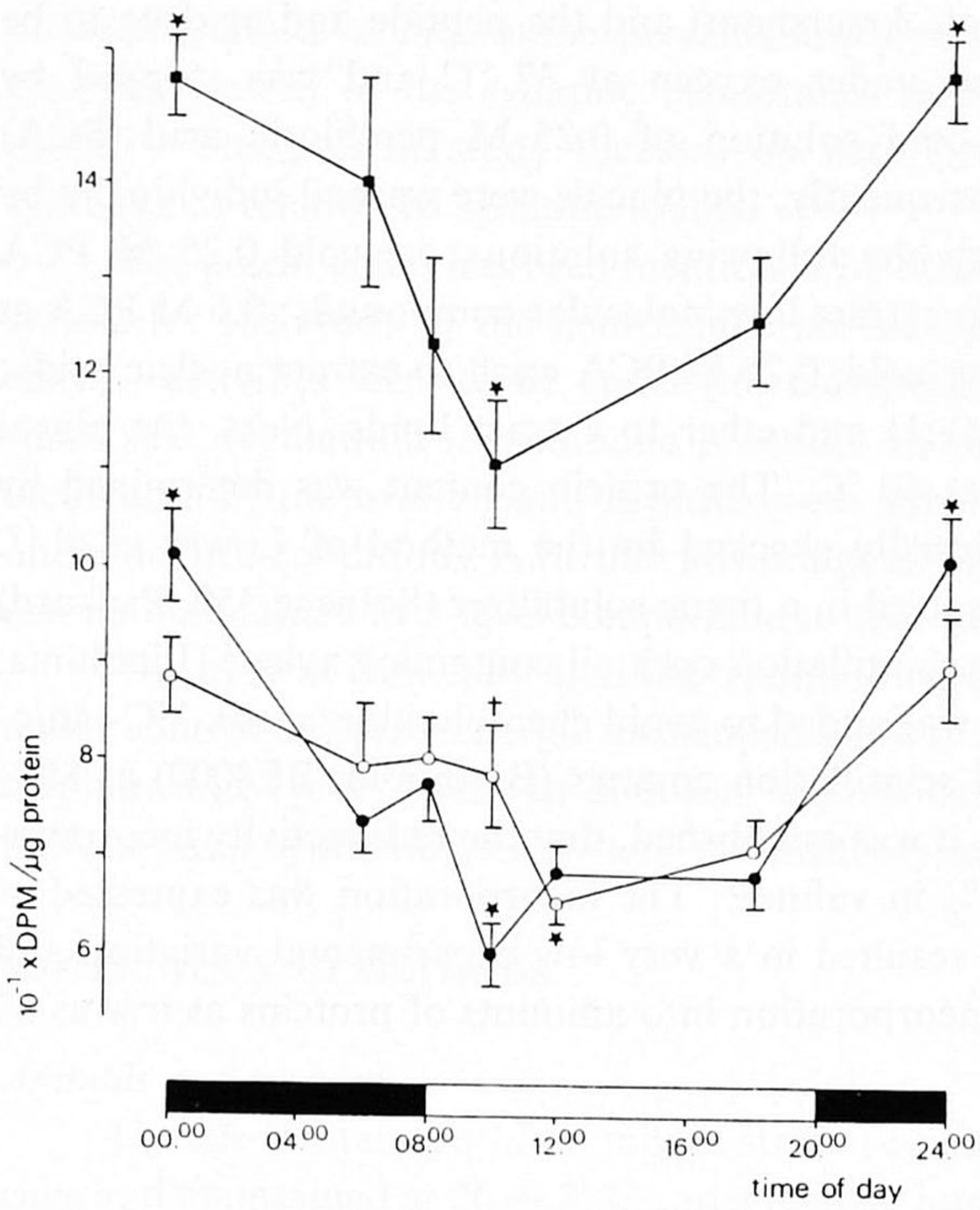

Fig. 2. Circadian rhythm. Rats were sacrificed at the times indicated on the horizontal axis and pineals were dissected. The animals were used either intact (-●-) or sham operated (data not shown, not different from intact), or 5 days after decentralization (-■-). Next, the incorporation of valine into proteins was measured under the standard conditions, i.e. over a 6 h period following 2 h of preincubation. The open circles (-○-) represent the data from pineals of intact rats incubated in the presence of ACTH (10^{-7} M). Bars represent mean ± S.E.M. (n = 6). *$P < 0.01$ 2-way analysis of variance indicates the time points that are different from the mean obtained by averaging data over the whole day. † $2P < 0.05$ Student's *t*-test; indicates the time points that are different as a consequence of $ACTH_{1-24}$.

was measured for periods of 2 h, starting the incubation at different times after preincubation, the amounts of radioactive valine incorporated into protein were similar over all periods (Fig. 1B). This was taken to indicate, that the overall incorporation rate remained constant over a period of at least 6 h after the start of the incubation.

When $ACTH_{1-24}$ was present during the incubation at a concentration of 10^{-7} M the incorporation of valine was higher (Fig. 1B). The difference relative to the control became significant over the last incubation period (4–6 h). Furthermore, taking all 3 incubation periods into account (Fig. 1B, mean), 2-way analysis of variance showed a significant increase due to ACTH. Therefore, a period of 6 h was chosen as a standard incubation condition. When cycloheximide (10^{-3} M), an inhibitor of protein synthesis, was present during the incubation, the incorporation of radioactive valine was decreased to 0.1 % of the control and no effect of ACTH (10^{-7} M) could be demonstrated (data not shown). This complete inhibition by cycloheximide showed that the present method measured changes in pineal protein synthesis.

From the mean valine-incorporation over 2 h (Fig. 1B, 22 dpm/μg protein) and using the previously determined amount of valine in the proteins (450 nmol valine mg protein[23]) we calculated a protein synthesis rate of 2.2 %/h. This is the same value as calculated by Dunlop and Lajtha for the in vivo rate[6].

Circadian rhythm in valine-incorporation and in the sensitivity to $ACTH_{1-24}$

The incorporation of valine into pineal proteins appeared to be highly dependent on the time of the day at which the pineals were dissected (Fig. 2) when the incorporation was measured under the standard incubation conditions (see above). A maximum incorporation was observed at midnight — i.e. the middle of the dark period — whereas a sharp minimum appeared to exist at 10.00 h, i.e. 2 h after the start of the light period.

When the pineals had been decentralized 5 days before, the level of incorporation appeared to be about twice as high at all times of the day, when compared to intact or sham operated rats (Fig. 2). Thus, decentralization considerably increased pineal protein synthesis, leaving its rhythmicity unaffected.

Circadian influences on the sensitivity to $ACTH_{1-24}$ were screened at a concentration of 10^{-7} M of the peptide. This dose effectively stimulated the valine-incorporation at 10.00 h (Fig. 1B). Fig. 2 (open circles) also shows that the sensitivity of pineal protein synthesis to ACTH is highly dependent on the time of the day at which the pineals were dissected, i.e. a significant stimulation by ACTH (10^{-7} M) was

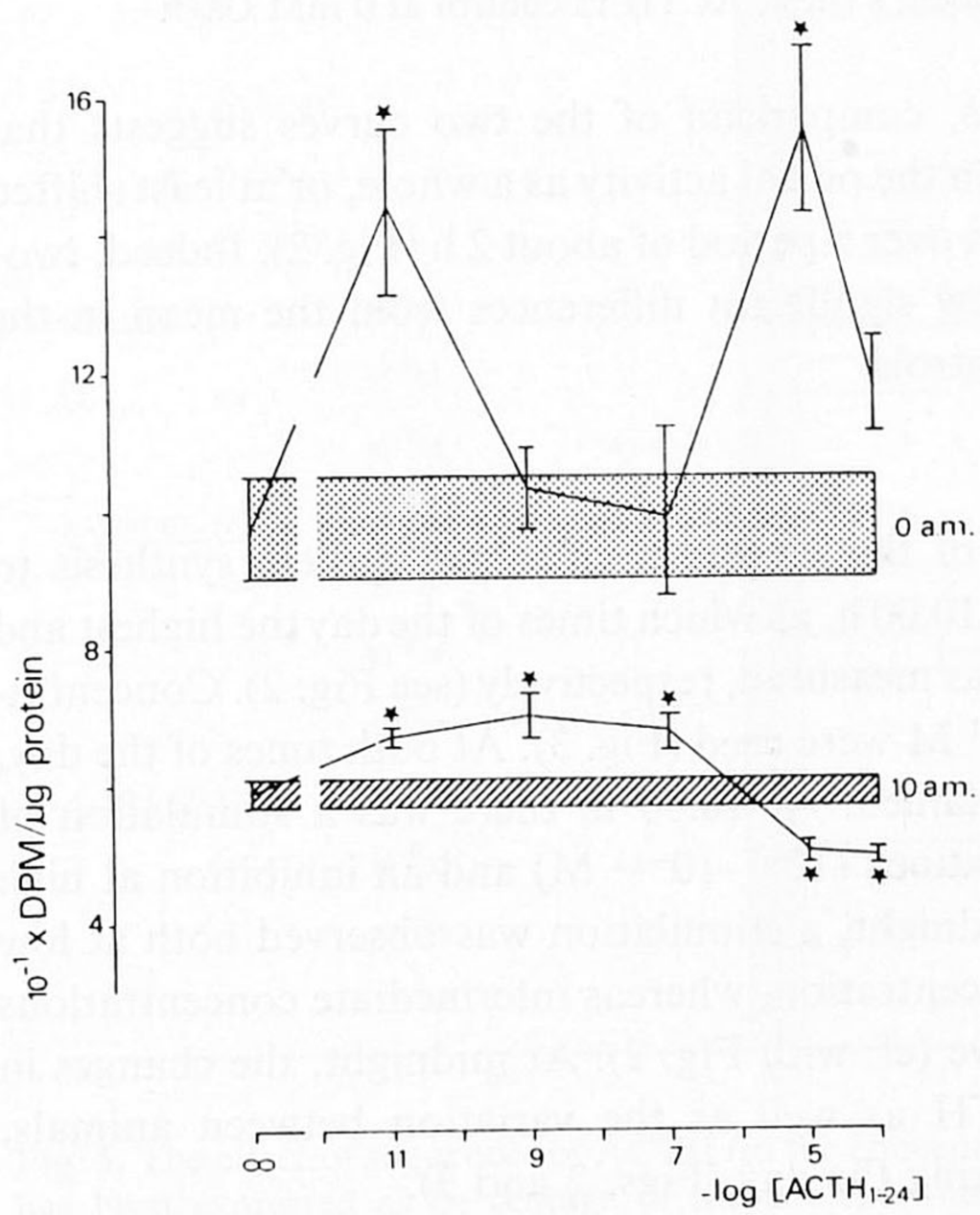

Fig. 3. Dose–response curve of the sensitivity to $ACTH_{1-24}$ at 24.00 h and 10.00 h. Pineals were dissected at 24.00 h or 10.00 h and preincubated for 2 h. $ACTH_{1-24}$ was added at time 0 of incubation. Bars represent mean $\pm$ S.E.M. (n = 6). * $2P < 0.01$ Student's *t*-test, ACTH vs control.

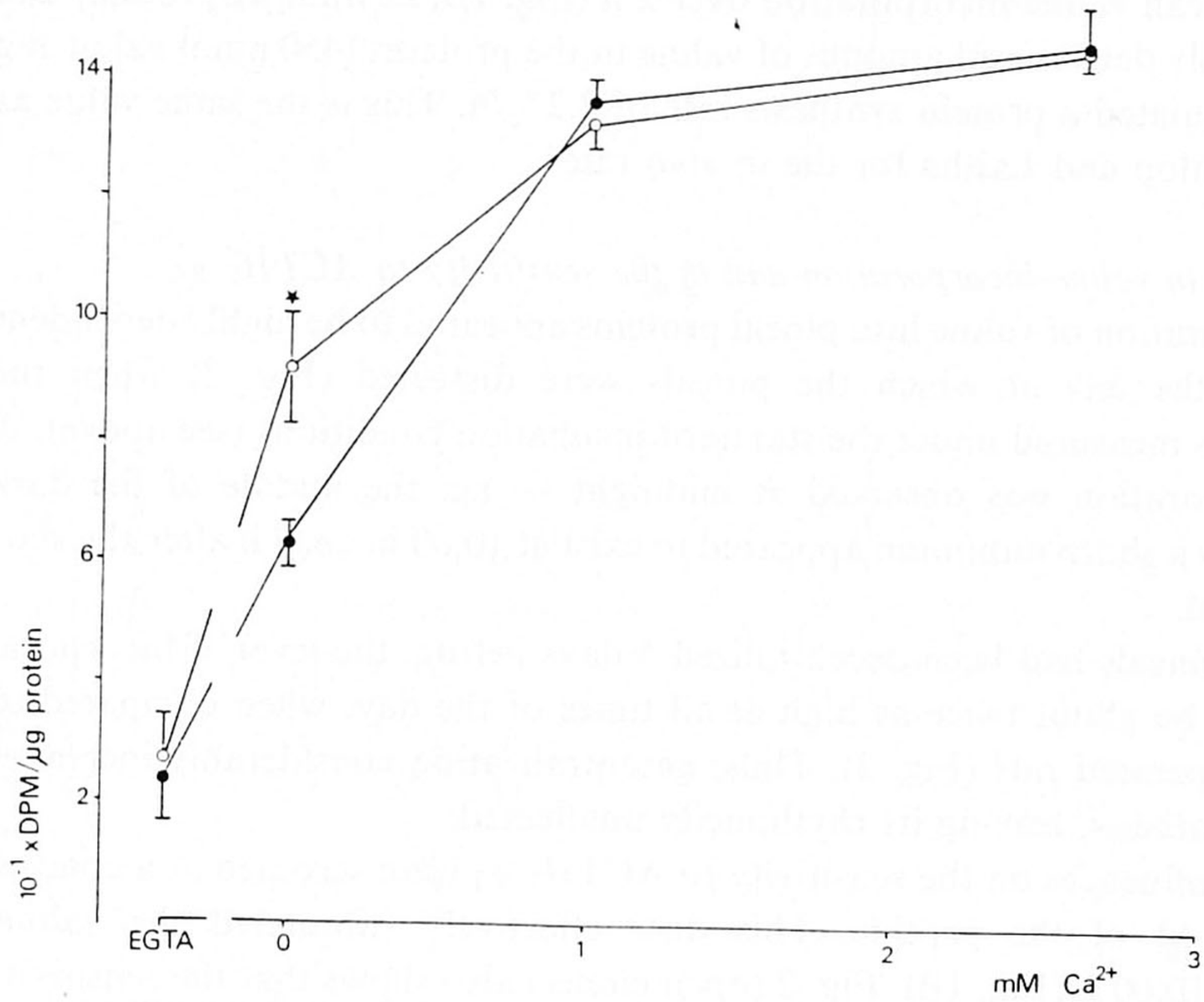

Fig. 4. Ca^{2+}-dependency of pineal protein synthesis and its stimulation by $ACTH_{1-24}$ (10^{-7} M) at 10.00 h. The incorporation of [1-^{14}C]valine and the effect of ACTH were measured over a 6 h period of incubation. Bars represent mean ± S.E.M. (n = 6) for control (closed circles) and $ACTH_{1-24}$ (10^{-7} M) (open circles). *$0.01 < 2P < 0.025$ Student's *t*-test, ACTH vs control at 0 mM Ca^{2+}.

only found at 10.00 h. Nevertheless, comparison of the two curves suggests that ACTH flattened out the rhythmicity in the pineal activity as a whole, or at least shifted the peaks in the incorporation pattern over a period of about 2 h (Fig. 2). Indeed, two-way analysis of variance revealed few significant differences from the mean in the presence of ACTH, compared to controls.

Dose–response curves for ACTH

A more detailed investigation of the sensitivity of pineal protein synthesis to ACTH was performed at 24.00 h and 10.00 h, at which times of the day the highest and the lowest level of protein synthesis was measured, respectively (see Fig. 2). Concentrations of $ACTH_{1-24}$ from 10^{-4} to 10^{-11} M were used (Fig. 3). At both times of the day, a biphasic dose–effect curve was obtained. At 10.00 h, there was a stimulation of valine-incorporation at low concentrations (10^{-7}–10^{-11} M) and an inhibition at high concentrations (10^{-5}–10^{-4} M); at midnight, a stimulation was observed both at low (10^{-11} M) and at a high (10^{-5} M) concentration, whereas intermediate concentrations of ACTH (10^{-7}–10^{-9} M) were inactive (cf. with Fig. 2). At midnight, the changes in incorporation rate induced by ACTH as well as the variation between animals, appeared to be much higher than during the day (Figs. 2 and 3).

The role of calcium (Ca^{2+})-ions.

Calcium ions have been reported to be essential both in protein synthesis in vitro

and for the effects of peptide hormones[8,9,27,36]. Fig. 4 shows the calcium-dependency of pineal protein synthesis in vitro with or without $ACTH_{1-24}$ (10^{-7} M). The presence of calcium in the medium (1.0 or 2.6 mM) resulted in a two-fold increase of valine-incorporation whereas EGTA (ethylene glycol-bis-(aminoethyl ether) N,N[1]-tetra acetic acid, 2 mM) decreased the activity to 40% of control. In this experiment, $ACTH_{1-24}$ (10^{-7} M) stimulated the activity when no exogenous calcium was supplied to the medium and no endogenous calcium ions were removed by EGTA.

Structure-activity relationship of the effect of ACTH

A structure–activity study was performed to relate the present effects of $ACTH_{1-24}$ on pineal protein synthesis to its known behavioral and neurochemical activities. In Fig. 5, a summary of several experiments is given. The effects were expressed relatively to their controls (100%).

Splitting the $ACTH_{1-24}$-molecule into the sequences (1–10) and (11–24) inactivated the peptide. Furthermore, the structure–activity relationship pointed to an active sequence in the region of (5–16).

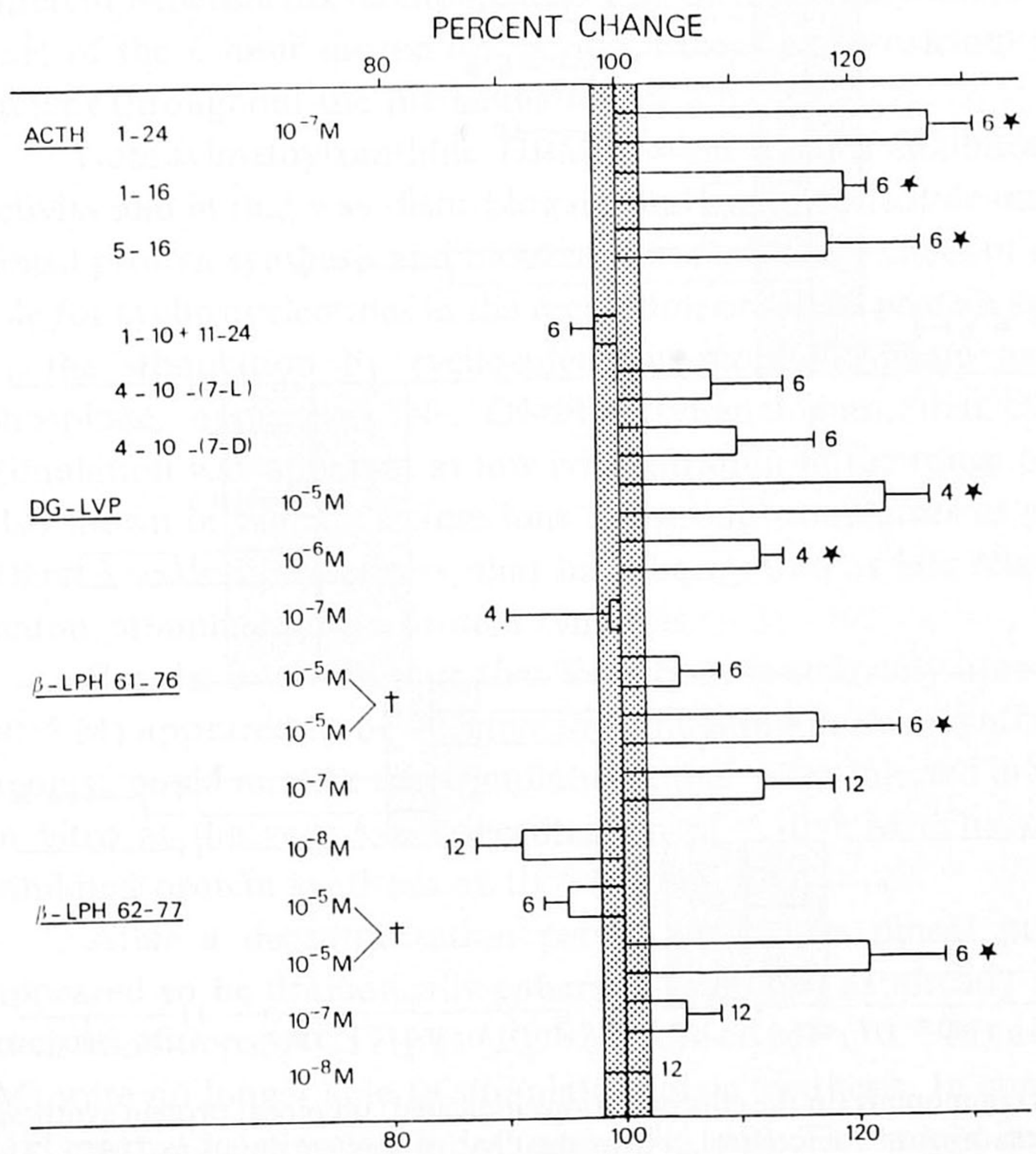

Fig. 5. The effect of sequences of ACTH (in the concentrations indicated) on pineal protein synthesis has been expressed as percentage of the control where the control was set equal to 100%. The dissection was performed at 10.00 h; incubation time with the peptides and [1-^{14}C]valine was 6 h. Bars represent mean ± S.E.M. with the number of incubations indicated. The shaded area indicates the variation in the controls. * $2P < 0.05$ Student's *t*-test. † The data represent two separate experiments; at other concentrations the data were combined.

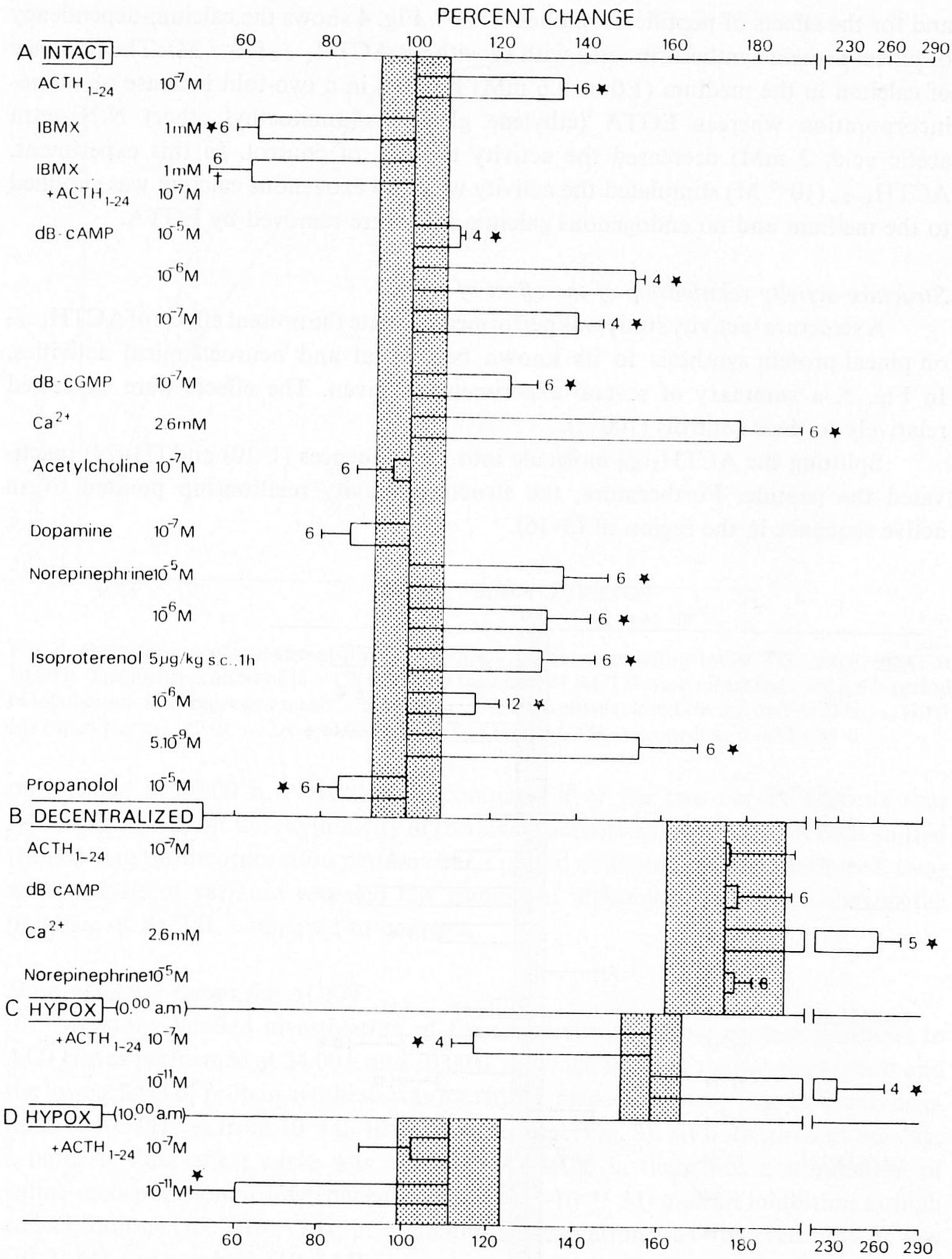

Fig. 6. The effects of various compounds (in the concentrations indicated) on pineal protein synthesis have been expressed as percentages of the control, where the control was set equal to 100%. The dissection was performed at 10.00 h except for one group of hypophysectomized rats as indicated. Incubation time with the compounds and [1-^{14}C]valine was 6 h. Bars represent mean ± S.E.M. with the number of incubations indicated. The shaded areas indicate the controls and their variation. The effect of the various compounds in pineals of decentralized and hypophysectomized rats were evaluated as compared to their untreated, operated controls. * $2P < 0.05$ Student's t-test. † not significant to IBMX, per se.

In addition to ACTH desglycinamide lysine vasopressin (DG–LVP, Fig. 5) also showed considerable activity in modulating pineal protein synthesis. It should be noted, that this peptide is devoid of classical pressor and antidiuretic activity.

Two sequences of β-lipotropin (β-LPH, Fig. 5) which have been previously shown to have activity on protein synthesis in a cell-free system of brain stem (Schotman, in preparation), were tested at several concentrations in the present system. These peptides, α- and dTγ-endorphin, evoked a small increase in protein synthesis at the highest concentration tested (10^{-5} M) in only one of the two experiments that have been performed.

Characterization of the sensitivity of pineal protein synthesis to various drugs

The relation between neural and hormonal control of pineal protein synthesis and the possibility that second messengers are mediators for these influences was explored. For this purpose, valine-incorporation was measured in the presence of the appropriate drugs.

In Fig. 6, a compilation has been made from the data of a large number of different experiments (compare also Fig. 5). The compounds were each added at the start of the 6 hour incubation period, except when calcium was added, it was also present throughout the preincubation of 2 h.

Isobutylmethylxanthine (IBMX) — a known inhibitor of phosphodiesterase activity and in that way disturbing normal cyclic nucleotide metabolism[32] — inhibited pineal protein synthesis and blocked the stimulatory effect of $ACTH_{1-24}$ (Fig. 6A). A role for cyclic nucleotides in the regulation of pineal protein synthesis was also shown by the stimulation by cyclic-adenosine-monophosphate and cyclic-guanyl-monophosphate, applied as N^2, O^6-dibutyryl-analogues, that can enter the cell[32]. The stimulation was apparent at low concentration in the range of 10^{-6}–10^{-7} M. As was also shown in Fig. 4, calcium ions are strong stimulators of pineal protein synthesis. Thus, 3 second messengers, that have been more or less related to neurotransmitter action, stimulate pineal protein synthesis.

For the 3 transmitters that have been tested, only norepinephrine (at 10^{-5} and 10^{-6} M) appeared to be effective in stimulating protein synthesis. Isoproterenol, a β-agonist, could mimick this stimulation either when injected in vivo, 1 h before death or in vitro at the very low concentration of 5.10^{-9} M. The β-antagonist propanolol inhibited protein synthesis at 10^{-5} M (Fig. 6A).

After a decentralization period of 5 days pineal protein synthetic activity appeared to be dramatically enhanced (Fig. 6B) as already shown in Fig. 2. Under such conditions, $ACTH_{1-24}$ (10^{-7} M), dB-cAMP (10^{-6} M) and norepinephrine (10^{-5} M) were no longer able to stimulate protein synthesis. In contrast, calcium (2.6 mM) still exerted its stimulatory influence (Fig. 6B). This might imply, that more than one mechanism is involved in the presently reported, stimulatory effects on pineal protein synthesis.

Three weeks after hypophysectomy rats were used to examine whether pineal protein synthesis might have undergone changes in sensitivity to ACTH as a result of the ACTH depletion caused by the surgery. Compared to sham-operated controls,

hypophysectomy resulted in an increase in pineal protein synthetic activity at midnight (Fig. 6C) but not at 10.00 h (Fig. 6D). The sensitivity to $ACTH_{1-24}$, under these conditions, showed biphasic effects at midnight: a decrease at 10^{-7} M and an increase at 10^{-11} M (Fig. 6C). At 10.00 h the biphasic effect was no longer seen, only the very low concentration of 10^{-11} M resulted in a decrease (Fig. 6D).

DISCUSSION

The pineal gland was used to study the modulation of protein synthesis by neuropeptides in vitro. Metabolic processes in the pineal are under noradrenergic control in situ[1,2]. This situation can be maintained in vitro for at least several hours[33,34].

The experimental design included, successively, rapid dissection of the pineal, a preincubation period of 2 h to stabilize the preparation and incubation for up to 6 h with ACTH or other test substances in the presence of 1 mM [1-^{14}C]valine. The use of valine at this high concentration avoided the so-called precursor pool problems[6] by constantly flooding all pools with exogenous valine[6,22]. In addition this amino acid is known to label almost exclusively proteins, because it gives rise to few metabolites and those metabolites that have been formed have lost their carboxyl label[24]. In this way, the rate of incorporation was shown to be constant for at least 6 h (Fig. 1B). Additional evidence that only proteins were labeled in this system is that the incorporation is completely sensitive to cycloheximide. Therefore, the incorporation rate could be taken as an appropriate measure of the overall protein synthesis rate of pineal. The non-linear curve of Fig. 1A can be explained by the existence of classes of pineal proteins with various turnover rates, the shortest one being in the order of 2 h. No attempts were made to explore changes measured in the overall incorporation rate due to the constituting classes of proteins with rapid or slower turnover rates. The incubation period of 6 h appeared to be suitable to measure the modulation of protein synthesis by ACTH and other agents (Figs. 1 and 6). The calculated in vitro rate of protein synthesis of pineal was comparable to that in vivo[7,23], which places the in vitro findings in a physiological perspective.

In the pineal, many properties and metabolic processes show circadian[1,21] or even seasonal variations[18]. Accordingly, we find that protein synthetic activity in vitro exhibited a marked circadian rhythm (Fig. 2). The activity was found to be dependent on the time of the day that dissection took place and for that reason the in vitro incorporation rate may reflect that of the pineal in situ. A parallel situation was observed in previous studies on the release of corticotrophic activity measured by pituitary glands incubated in vitro[14]. Pineal protein synthesis in situ is, apparently, high during the night and shows a minimum a few hours after the onset of light (Fig. 2). Similar rhythms appeared to exist in the activity of serotonin-N-acetyltransferase (SNAT) and hydroxy-indole-O-methyl-transferase (HIOMT)[1]. Those rhythms could be related to changes in sympathetic nerve activity and norepinephrine turnover in the neurons that innervate the pineal[1,2,31] and to changes in binding sites and sensitivity of the postsynaptic β-receptor[1,21]. The rapid changes in SNAT and HIOMT activities

have been explained both in terms of induction at the transcriptional and translational level as well as direct activation[1,25,33]. Changes in pineal properties after denervation have been described in terms of supersensitivity of the β-adrenergic receptor due to reduced stimulation experienced by the gland[1,25]. The higher level of amino acid incorporation in vitro in pineals after denervation (Figs. 2 and 6) might thus reflect a change at the translational level resulting from an altered β-receptor sensitivity. However, the maintenance of the circadian rhythm superimposed upon the increase in activity after denervation implies that protein synthesis in pineals is not solely under control of sympathetic nerve activity. Endocrine influences also seem important[2,18,25].

Further evidence for the involvement of hormones is suggested by the finding that the sensitivity to neuropeptide-stimulation was highly dependent on the time of the day (Fig. 2). Dose–response curves for the effects of $ACTH_{1-24}$ (Fig. 3) suggest that at midnight, when basal protein synthetic rate attained its highest level (Fig. 2) a higher sensitivity to neuropeptide-stimulation existed (Fig. 3). A close correlation between basal activity and hormone-sensitivity was previously found for adenylate cyclase activity in the pineal and might be a common feature of receptor mediated processes[19]. The changes in sensitivity to ACTH are parallel with diurnal variations in ACTH and α-MSH levels observed in blood plasma and brain regions[14,20,28]. This might relate changes in sensitivity to different levels of the modulator; a similar correlation was described for the neurotransmitter norepinephrine[1] and the hormone testosterone[3].

Both at 10.00 h and 24.00 h, dose–response curves for ACTH have a biphasic shape. The responses to dB-cAMP, isoproterenol and to hypophysectomy also showed such a tendency (Fig. 6). Biphasic, dose-dependent responses to ACTH have been described before for behavioral activity mediated by norepinephrine[12], for brain adenylcyclase activity[32], for phosphorylation of synaptic plasma membrane proteins[38] and for cell-free protein synthesis in brain[27]. Thus, this phenomenon might constitute an intrinsic feature of hormonal, neurotropic action and might indicate a role for more than one regulatory site used by ACTH (see below).

Calcium (Ca^{2+}) is a common mediator for all processes mentioned above and also for the sensitivity of these processes to modulation by ACTH (Figs. 3 and 6). Pineal protein synthesis was stimulated by the presence of calcium in the medium even after denervation. However, in the absence of calcium the stimulation by $ACTH_{1-24}$ (10^{-7} M) was maximal (Fig. 3). Previously, in the adrenals a similar calcium dependency has been observed on protein synthesis, steroidogenesis and the ACTH-induced stimulation of these two processes[8,9]. Similarly, in brain, a calcium dependency has been shown for cell-free protein synthesis, adenylate cyclase activity, synaptic plasma membrane phosphorylation and their modulation by ACTH[27,32,35,36].

The specificity of the effects brought about by $ACTH_{1-24}$ may follow from the ineffectiveness of the fragments 1–10 and 11–24 and their equimolar combination (Fig. 5). The active sequence within the $ACTH_{1-24}$-molecule appeared to be within 5–16. It should be noted that this sequence is devoid of classical adrenocorticotropic and lipotropic activity in vivo[4]. Similar structure–activity relationships have previously been found

for the effects of ACTH on metabolism of cyclic nucleotides[32] in brain, phosphorylation of synaptic plasma membrane proteins[36] and induction of excessive grooming behavior[11]. Once again this indicates an interrelationship between these phenomena and their modulation by ACTH.

Stimulation of pineal protein synthesis was not strictly limited to analogues of ACTH. Desglycinamide lysine-vasopressin (DG–LVP), a naturally occurring analogue of vasopressin[4], exerted a stimulatory effect as well (Fig. 5).

It is already known from the literature that the pineal is under the feed back control of reproductive hormones[3]. This prompted Cardinali to make the statement that the pineal, being itself a source of hormones[18], acts both as a neuroendocrine gland and as an 'endocrine-neural' transducer[1]. The present data indicate that hypophyseal hormones like ACTH, vasopressin and related peptides, are able to modulate pineal protein synthesis, directly (Figs. 1, 2, 3 and 5), even when applied in concentrations within the physiological range[20,28,29]. The extent of this modulation was comparable to that observed from changes in innervation (Figs. 2 and 3).

In order to explore the relation between neural and hormonal influences, catecholaminergic agents and second messengers were tested under different neural and hormonal states (Figs. 6A,B and C). The present observations combined with data from the literature can be brought into one scheme (Fig. 7), in which hypophyseal principles (e.g. ACTH like peptides) trigger changes in the state of overall protein synthesis in the pineal through β-receptor mechanism. This scheme is essentially based on the following observations: (1) modulation of pineal protein synthesis by ACTH and norepinephrine was found after denervation (Fig. 6B); (2) The dose-dependent stimulation of protein synthesis by the agonist isoproterenol and the inhibition by the antagonist propanolol; (3) Selective sensitivity of protein synthesis for norepinephrine (Fig. 6A); (4) the existence of a circadian rhythm both in protein synthesis and its sensitivity to ACTH (Figs. 2 and 3). β-Receptor mediation was proposed for changes in pineal adenylate cyclase activity earlier[19]. The inhibition of the expression of the ACTH-effect by IBMX (isobutylmethylxanthine) and the dose-dependent sensitivity of protein synthesis to dibutyryl cyclic AMP, indeed suggests an intermediate role for cyclic AMP in the modulation by ACTH[32]. An intermediate role for cyclic AMP was previously described for the influence of norepinephrine and β-receptor stimulation on the enzyme activities in pineal of SNAT (serotonin N-acetyltransferase) and HIOMT (hydroxy indole methyl transferase)[1,16,25,33].

So far, Fig. 7 schematizes the neural and circadian control of pineal protein synthesis. However, why in the present study denervation led to ineffectiveness of $ACTH_{1-24}$, but calcium-sensitivity was not influenced (Fig. 6B) remains to explain. Loss of sensitivity of pineal protein synthesis to gonadal hormones has been previously reported under similar conditions[3]. It may be that as proposed for the cyclic nucleotides before[19] both pre- and postsynaptic mechanisms are in operation. Hormones like ACTH might interact mainly with presynaptic receptors, whereas calcium may also act directly at the postsynaptic level. Such a view is also supported by our previous results on the modulation by ACTH of a calcium dependent phosphorylation of a (pre)synaptic phosphoprotein (B-50)[15,35,36] on the one hand, and

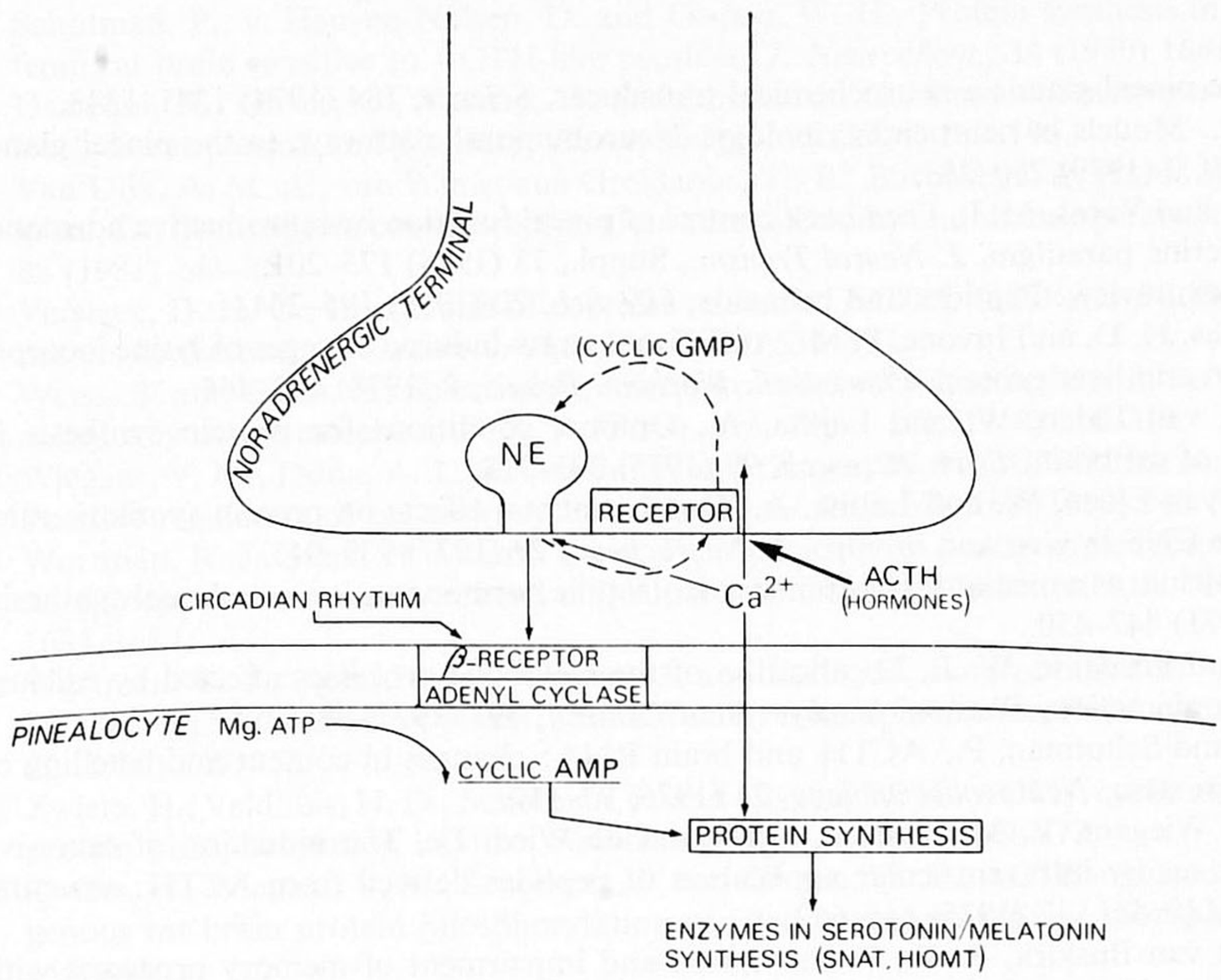

Fig. 7. Schema for the control of pineal activity by hormonal, neural and endogenous (i.e. circadian) influences.

the sensitivity to calcium of brain protein synthesis in a cell-free system on the other hand[27]. In any case, the present data suggest that there exists a neural control over pineal postsynaptic protein synthesis via β-adrenergic mechanisms being susceptible to both neuroendocrine and neuropeptide regulation. However, the pineal might not be unique among neural structures, in this respect[2]. Apparently, many parallel mechanisms exist in peripheral target organs[8,9] and in the rest of the brain[22,26,27,32,36] including behavioral processes[4,11,12]. Neural control over postsynaptic protein synthesis might constitute a factor in plastic changes in the nervous system. Endocrine stimulation at that level has its parallel in the well documented trophic effects of hormones on their peripheral target organs.

At present, it is unclear whether the effective peptides are released from the pituitary or whether the observed changes are brought about by ACTH-like peptides recently localized in the pineal[3,18]. However, the dramatic effects of hypophysectomy (Fig. 6C and D) seem to favor an important role for circulating, hypophyseal principles.

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