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Influence of melatonin on the process of protein and/or peptide secretion in the pineal gland of the rat and hamster

An in vitro study

Chandana Haldar-Misra[1]* and Paul Pévet[1,2]

[1] The Netherlands Institute for Brain Research, Amsterdam, The Netherlands;
[2] Department of Anatomy, University of Amsterdam, Amsterdam, The Netherlands

Summary. The hypothesis that melatonin (aMT) is implicated in the regulation of the secretory process involved in the synthesis and release of protein/peptide "hormones" of the pineal organ has been tested in the present study by the use of an in vitro system in two mammalian species. In these species the secretory process studied is characterized by either large (hamster) or very small (rat) numbers of granular vesicles.

In both species, aMT clearly participates in the regulation of the process of protein/peptide secretion. However, the effect of aMT varies with the presence of noradrenaline (NA) in the medium and is not identical in both species. Melatonin, in the absence of NA, induces the formation of granular vesicles by the Golgi apparatus in pinealocytes of the rat but not in those of the hamster, while in the presence of NA, aMT provokes a decrease in the number of these vesicles in both species.

The present experiments show (i) that the pineal is one of the target organs for aMT, and (ii) that aMT is implicated in the control of protein/peptide secretion in the pineal organ.

Key words: Pineal organ – Melatonin – Pineal protein secretion – Rat – Hamster

It is now evident that, in principle, the mammalian pineal gland is involved in long-term adaptation of certain functions (such as reproduction) to environmental conditions (Pévet 1976; Hoffmann 1979, 1981; Reiter 1973,

Send offprint requests to: Dr. Paul Pévet, Netherlands Institute for Brain Research, IJdijk 28, 1095 KJ Amsterdam, The Netherlands

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* IBRO/UNESCO fellow; present address: C. Haldar-Misra, Department of Zoology, Banaras Hindu University, Varanasi 221005 UP India

1980). The mechanism by which the pineal acts on the gonadal axis is, however, not yet known.

Recently, it has been hypothesized that the different 5-methoxyindoles synthesized by the pineal gland, the retina, the Harderian gland, and the intestine might be implicated in a system enabling the pineal gland and some other structures of the brain to perceive, differentiate and integrate environmental information (e.g., photoperiod, temperature, food supply). In response, the pineal may then synthesize and release protein/peptide hormone(s), which may in turn act on the reproductive axis (Pévet et al. 1981; Pévet and Haldar-Misra 1982). In this concept, in addition to its possible physiological role at the level of the hypothalamo-hypophyseal-gonadal axis (for review, see Reiter 1980; Cardinali 1981; Hoffmann 1979, 1981), melatonin (aMT, cf. Smith 1982) may be concerned with the regulation of the secretory processes involved in the synthesis and release of protein peptide hormone(s). Such a direct effect of aMT on the secretory activity of the pineal organ, which had been suggested already by Quay in 1974, has been demonstrated to occur in the mouse in vivo (Benson and Krasovich 1977) and in vitro (Haldar-Misra and Pévet 1983).

In the mouse, the process of protein/peptide secretion is characterized by the formation of a large number of granular vesicles (GV). In general terms however, this process, as can be judged from the number of GV in the pinealocytes, appears to be more or less well-developed depending on the mammalian species examined (for review, see Pévet 1979, 1981, 1983). It therefore appeared important to study this effect of aMT in the pinealocytes of other mammalian species, characterized – in untreated animals – by the presence of either large (hamster) or very small (rat) numbers of GV. The in-vitro model system developed by Haldar-Misra and Pévet (1982) was used for this study. Since in previous experiments (Haldar Misra and Pévet 1982) it had been shown that noradrenaline (NA) is basically involved in the regulation of this process of protein/peptide secretion either directly or indirectly via the NA-induced formation of another compound, all experiments were performed in parallel (i) with a noradrenaline-free medium (NA-free medium), and (ii) with a medium containing noradrenaline (NA-containing medium).

Materials and methods

1. Animals

Sixteen young adult male hamsters (± 70 g) and eight young adult male rats (± 130 g) obtained from TNO, Zeist, The Netherlands, were used for the present study. After their arrival at the laboratory the hamsters were maintained for 4 weeks in light, from 4 a.m. to 6 p.m. (14 L/10 D), at 20° C in constant humidity and received tap water and food ad libitum. The rats were kept under the same conditions, except that they were subjected to a 12 L/12 D schedule.

2. Organ culture technique

After sacrificing the animals by decapitation (hamsters in February, rats in March), the pineal glands were aseptically removed. Pineal explants (hamster, the entire organ; rat, one half

Table 1. Experimental protocol used for the study of the effects of 15-h application of melatonin (aMT) on explants of rat and hamster pineal organs

Experimental conditions	Days 1, 2, 3	Day 4 (between 15.00–17.00 h)	Day 5 (between 9.00–10.00 h)
A. With NA-free medium			
Control (4)	medium	renewal of the medium +solvent	fixation
Melatonin (4)	medium	renewal of the medium +aMT	fixation
B. With NA-containing medium:			
Control (4)	medium	renewal of the medium +solvent	fixation
Melatonin (4)	medium	renewal of the medium +aMT	fixation

Solvent: 5 µl ethanol in 100 µl distilled water/10 ml medium (x) Number of pineals (hamster) or of explants (rat) per experimental group

of the organ) were cultured in complete darkness for 5 days, an optimal time for the process studied (Haldar-Misra and Pévet 1982), the medium being renewed at day 4. The details of the technique used as well as the composition of the medium have been described previously (Haldar-Misra and Pévet 1982).

3. Experimental protocol

The protocol used to study the effect of 15-h melatonin application is presented in Table 1.

Melatonin (f.c. 0.02 mM) and L-noradrenaline bitartrate (f.c. 0.1 mM) obtained from Sigma Chemical Co., St. Louis, Miss., USA) were added to the medium immediately before use.

4. Fixation and quantification

After incubation the pineal explants were fixed by immersion with 2.5% glutaraldehyde + 1% paraformaldehyde in 0.2 M Na-cacodylate buffer, pH 7.3, and postfixed in 1% omium tetroxide in 0.2 M Na-cacodylate buffer, pH 7.3, for electron microscopy. Ultrathin sections were stained with uranyl acetate and lead citrate (Reynolds 1963; Venable and Coggeshall 1965). Observations were carried out with a Philips 200 electron microscope.

For quantification, a modification of the technique of Benson and Krasovich (1977) described in detail by Haldar-Misra and Pévet (1982) was used. Photographs (50 for one explant) were made at regular intervals from the explants. The GV were then quantified in the pinealocytes of the rat (very low number) and in two different portions of the pinealocytes (the perikaryon and the endings of the cell processes) of the hamster. The respective areas in the electron micrographs were calculated by the use of a Manual Optical Picture Analyser (MOP system). The results were expressed as the number of GV/50 μm^2 cytoplasmic area and statistically analyzed (Student's *t*-test, considering $p < 0{,}05$ as significant).

Results

1. Rat

After 5 days of culture the pinealocytes resemble morphologically the characteristic elements of the pineal organ in vivo (for reference, see Karasek

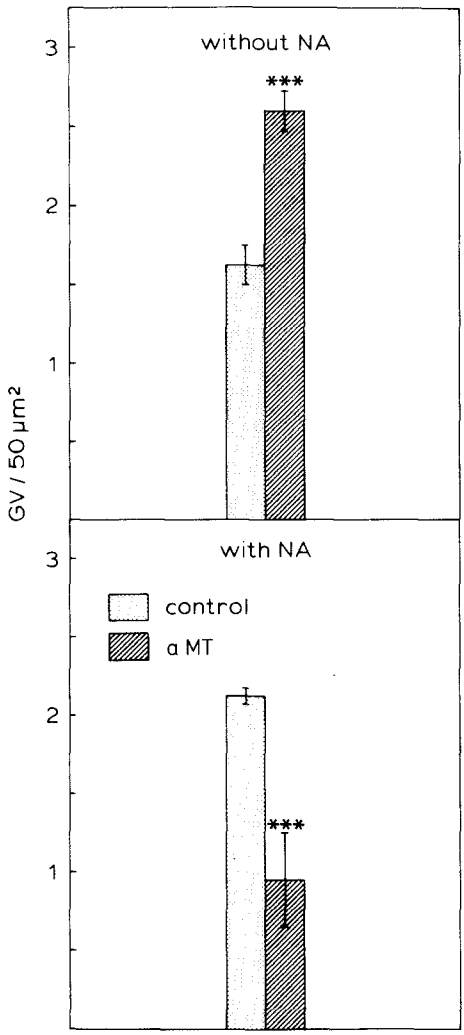

Fig. 1. Effect of application of melatonin (aMT) for 15 h on the number of granular vesicles (*GV*) observed in rat pinealocytes after 5 days of culture in noradrenaline-free medium or in adrenaline-containing medium
*** $p < 0{,}001$

1971). Numerous lipid droplets and well-developed Golgi complexes associated with numerous clear vesicles were commonly observed. As in the in vivo situation, the number of GV present in the cell is very small. However, in this experiment, as in an earlier one (Haldar-Misra and Pévet 1982), the number of GV increases in explants cultured in a NA-containing medium (Fig. 1).

Addition of aMT to the NA-free medium induces a significant increase in the number of GV (Fig. 1). Interestingly, this increase is higher than that obtained after NA application (Fig. 1). Considering the other cell organelles that could be implicated directly or indirectly in the process of protein secretion, some changes were observed. More specifically, numerous large vesicles (vacuoles?) of unknown origin, either scattered or clustered

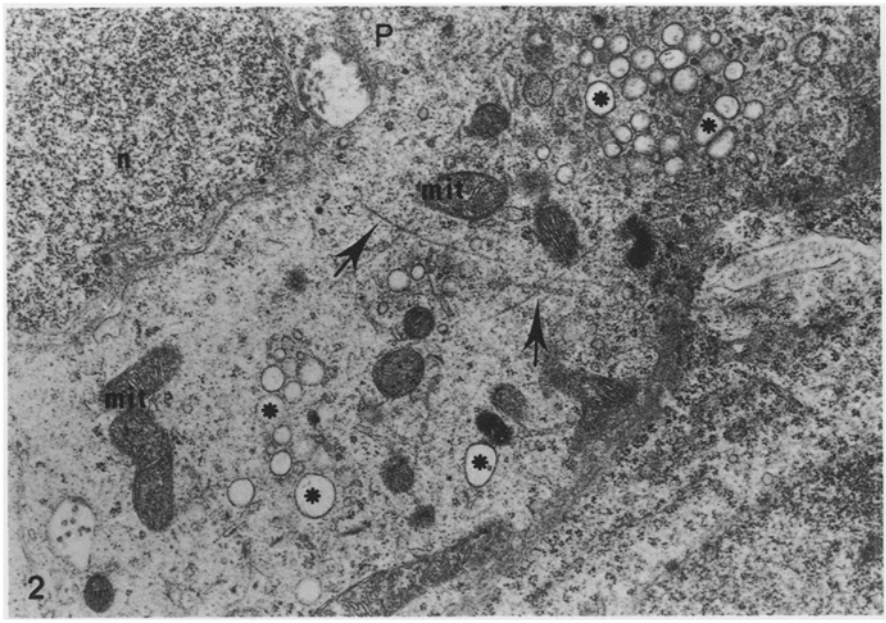

Fig. 2. Melatonin-induced formation of large, clear vesicles (*) in rat pinealocytes (*P*) cultured in noradrenaline-free medium; *mit* mitochondria; *n* nucleus; *arrows* microtubules. ×22800

in the perikaryon, were found (Fig. 2). In some of these elements a very small amount of electron-dense material was present, but most often they were apparently empty (Fig. 2).

In the presence of NA, aMT has an opposite effect, i.e., inducing not an increase but a decrease in the number of GV. Interestingly, under these conditions the number of GV is smaller than in pinealocytes cultured in NA-free medium. NA by itself, in this in vitro system, stimulates a hypertrophy of the Golgi complex and an increase in the number of parallel cisternae of the RER and of vesicle-crowned rodlets (VCR). Addition of aMT provokes in this "activated" system formation of a larger number of lysosomal structures.

2. Hamster

Essentially, in vitro as well as in vivo (Clabough 1970), a well-developed Golgi complex and a large number of GV are characteristic of hamster pinealocytes. Moreover, numerous mitochondria, cisternae of the rough (RER) and smooth (SER) endoplasmic reticulum, microtubules, and VCR are present. In the in vitro system, the activity of the cells depends of the presence or absence of NA in the medium. NA provokes a decrease in the number of GV in the perikaryon and an increase in the ending of the process (Fig. 3), a result that has already been interpreted as a consequence of the activation of protein/peptide secretion (Haldar-Misra and Pévet 1982).

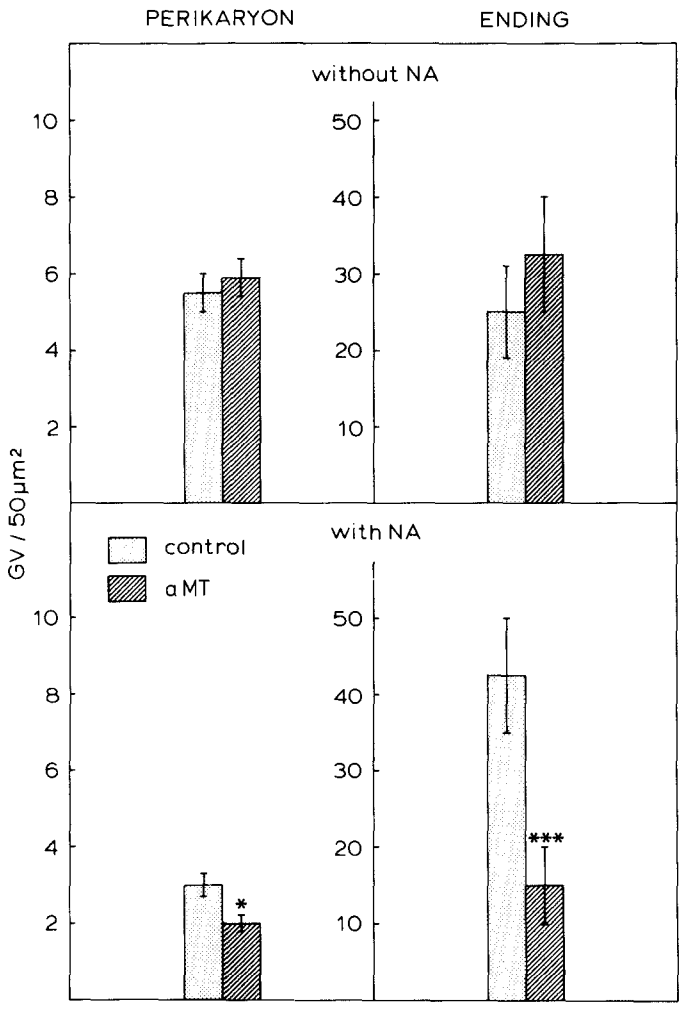

Fig. 3. Effect of application of melatonin (aMT) for 15 h on the number of granular vesicles (*GV*) observed in the perikaryon and the endings of processes of hamster pinealocytes after 5 days of culture in noradrenaline-free medium or in noradrenaline-containing medium
* $p < 0{,}05$; *** $p < 0{,}001$

When cultured in NA-free medium, aMT appears to have no effect on the process studied (Fig. 3), or on the other cell organelles. In contrast, when tested on pinealocytes cultured in NA-containing medium, aMT significantly decreased the number of GV in the perikaryon as well as in the endings of the processes (Fig. 3). In the endings, parallel with the decrease in number of GV, an increase in number of small, clear vesicles was observed. Regarding the other cell organelles, it appears also that aMT in the presence of NA induces a proliferation of the SER (Fig. 4). An increase in the number of VCR, especially in the endings, was also noted.

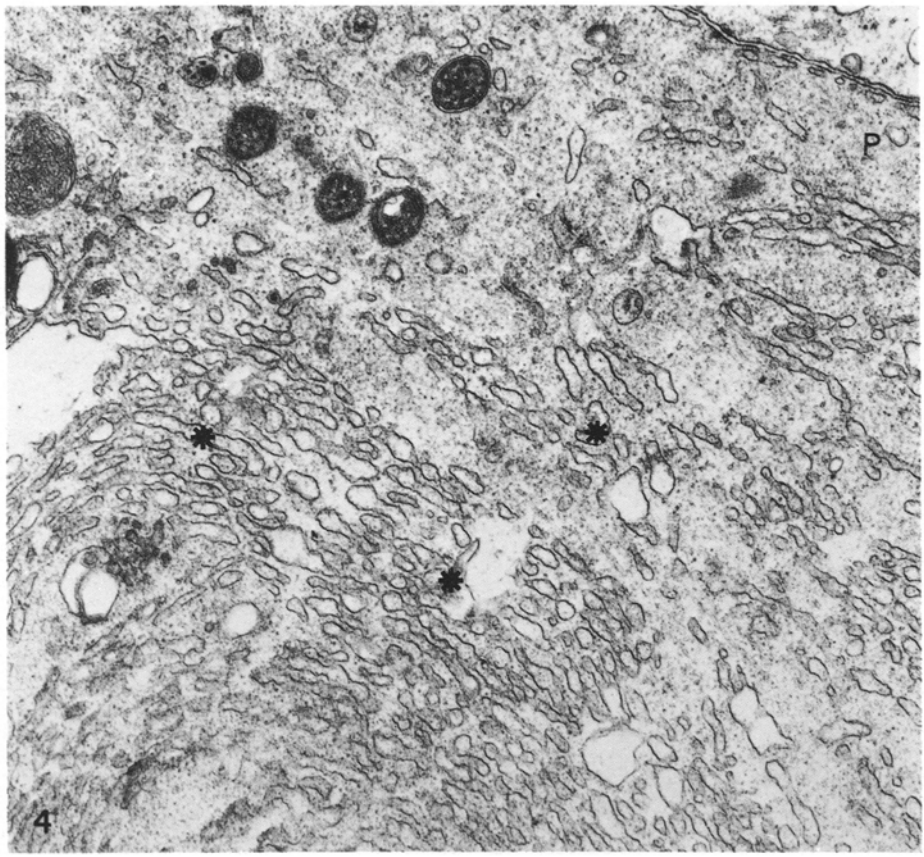

Fig. 4. Melatonin-induced proliferation of smooth endoplasmic reticulum (*) in hamster pinealocytes (*P*) cultured in noradrenaline-containing medium. × 26600

Discussion

In a previous study Haldar-Misra and Pévet (1983) have shown that in the pinealocytes of the mouse melatonin (aMT) is capable of influencing the process of protein/peptide secretion characterized by the formation of GV. The present results demonstrate clearly that this also holds true for the rat and the hamster, and thus support our working hypothesis (Pévet et al. 1981; Pévet and Haldar-Misra 1982), according to which melatonin may influence the production of protein/peptide factors in the pineal organ.

The present results indicate also that the influence of aMT on the process of protein/peptide secretion in the pineal organ varies with the presence of NA in the medium and is not identical in both species. In the absence of NA, melatonin clearly induces the formation of GV by the Golgi apparatus in pinealocytes of the rat, but not in those of the hamster. The results obtained in the presence of NA are more difficult to interpret; in both

species aMT provokes a decrease in the number of GV, a result that might be interpreted as an inhibitory effect on the process of protein/peptide secretion. However, the general ultrastructural aspect of the pinealocyte, characterized by the presence of numerous cisternae of the RER, an enlarged Golgi apparatus, and a large number of mitochondria, appears to be contradictory to this conclusion. Since the process studied is already stimulated by NA either directly of indirectly via the NA-induced formation of another compound. (Haldar-Misra and Pévet 1982), it might also be possible that the overstimulation following the addition of aMT causes an increase in the transport and release mechanism resulting in the observed decrease in the number of GV. This interpretation may be supported by the observation of the increased number of clear vesicles in the endings of the processes of hamster pinealocytes and may correspond to the enhanced activity of pinealocytes after administration of aMT as observed in the rat by Freire and Cardinali (1975).

These results raise a question concerning the nature of the effects of NA on the process of protein/peptide secretion as described by Haldar-Misra and Pévet (1982). Indeed, since in vitro addition of NA to the medium increases the rate of conversion of N-acetyl-serotonin to aMT (Klein and Weller 1973), it may be possible that the effects of NA are indirectly due to an NA-induced melatonin production. This concept is strongly supported by the fact that under the present experimental conditions both NA and aMT, when added to the medium, induce an increase in the number of GV. However, this may only hold true for the rat, since, in the hamster and in the mouse (Haldar-Misra and Pévet 1983), aMT alone – in contrast to NA – has no effect on the number of GV. Moreover, in the rat, hamster and mouse (Haldar-Misra and Pévet 1983) the effects of aMT in the presence of NA are opposite to those produced by NA alone. Consequently, although it cannot be excluded that under certain conditions a part of the effect of NA is indirectly due to aMT, both compounds may exert their own effects on the secretory process studied. Thus, apparently a very complex system regulates the process of protein/peptide secretion in the pineal organ (see also Haldar-Misra and Pévet 1983).

Melatonin also appears to be involved in the regulation of the activity of different cell organelles. In the rat, for example, aMT, in the absence of NA, stimulates the formation of a large number of large, clear vesicles (vacuoles?) (Fig. 2), which might reflect the formation of a different type of secretory material. Melatonin also induces in both species an increase in the number of VCR (also termed synaptic ribbons; see Vollrath 1981). This, again, demonstrates that VCR are active cell organelles, probably of great functional significance.

The increase in the number of cisternae of the SER observed in the hamster after application of aMT in the presence of NA, is difficult to interpret. However, if we recall that Romijn (1975), on the basis of pharmacological studies in the rabbit, hypothesized that the SER may be involved in indoleamine synthesis, and that Lin et al. (1975) arriving at a similar conclusion suggested that the enzyme hydroxyindole-O-methyltransferase

may occur in these cisternae, the present results might indicate that exogenously administered aMT acts on the synthesis of aMT (or other 5-methoxyindoles) in the pinealocyte itself. This interpretation, however, remains to be proven using chemical and immunocytochemical methods.

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