家蚕糸腺のサイクリックAMPおよびGMP量の変化とDNA合成

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Levels of cyclic AMP and cyclic GMP int he silk gland of *Bombyx mori*, in relation to DNA synthesis

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The concentration of cyclic AMP and GMP in the postereor silk gland were determined in both untreated silkworm larvae and those treated with the juvenile hormone analogue, methoprene. The levels of cyclic AMP and GMP increased gradually from the initiation period at the 5 th instar and reach a maximum of 75 pmol and 25 pmol in the control larvae, and 118 pmol and 25 pmol in the treated larvae, respectively. These increases of cyclic nucleotide levels corresponded with levels of DNA synthetic activity and were curtailed when DNA synthesis ceased. The correlation between increase of the two cyclic nucleotide levels and initiation of DNA synthesis was discussed.

With the exception of the molting stages, DNA synthesis in the posterior silk gland is maintained during larval groth, and is discontinued in the middle of the 5 th instar stage of silkworm larvae (Gillot and Daillie, 1968). This DNA synthesis is substantially affected by exogenous juvenoids (Kurata and Daillie, 1978).

In mammals, the multiplication of cultured cells (Short *et al.*, 1972 ; Liffeert, 1974) is affected by glucagon and insulin, and it has been suggested by Armato *et al.* (1978) that DNA-synthetic stimulation and the growth promoting effect of pancreatic hormones in the cultured cells are mediated via viclic adenosine 3′, 5′-monophosphate (cyclic AMP). Furthermore, it has been shown that the addition of equimolar mixtures of dibutyryl cyclic AMP and dibutyryl cyclic 3′, 5′-guanosine monophosphate to neonatal rat hepatocytes induces DNA synthesis and that by the addition of high, non-physiological concentrations of cyclic 3′, 5′-guanosine monophosphate (cyclic GMP), substantial increases in DNA synthesis could be induced in resting fibroblasts (Armato *et al.*, 1981).

Studies of cyclic nucleotides in the silkworm have been few, and only a series of studies on nucleotide cyclases, controlling cyclic nucleotide concentrations in cells, in the fat body of the silkworm has been carried out by Morishima (1980, 1981).

Here, I have dealt with the correlation between quantitative changes in interacellular cyclic AMP and cyclic GMP concentrations and DNA synthetic activity in the posterior silk gland of control and JH-treated larvae of the silkworm.

Materials and Methods

Silkworms and silk glands

Hybrid larvae produced from crosses between Nichi 124 and Shi 124 strains of *Bombyx mori* were reared on mulberry leaves at 25℃ during the 4 th and 5 th instar. Posterior silk glands for assay of cyclic AMP and GMP and DNA were dissected out periodically every day from

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the last day of the 4 th instar to the end of the 5 th instar, forzen in the acetone dry-ice bath, and stored at -20℃ in a deepfreezer until used.

Extraction and determination of cyclic AMP and GMP

Frozen silk glands were homogenized with a glass homogenizer in 5 % trichloroacetic acid (TCA) solution and precipitated proteins were removed by centrifugation at 3,000 rpm for 15 min. at 5 ℃. Supernatants were extracted 5 times with 4 volumes of water-saturated ether to remove the TCA. The final pH was 7.5. The aqueous solution was lyophilized and used for assay. Cyclic AMP and GMP assay kits (Radio-chemical center, Amersham, England) were employed for determination of each cyclic nucleotide.

DNA extraction and determination

DNA extraction was performed by the procedure of Schmid and Thanhauser (1945). The determination of DNA was performed by a modification of dephenilamine method (Burton, 1956).

Measurement of DNA synthetic activity

DNA synthetic activity was measured by the incorporation of tritiated thymidine ([^{3}H]-TDR) or tritiated thymidine triphosphate ([^{3}H]-TTP) into DNA of the silk gland *in vitro* for one hour as described previously (Kurated and Daillie, 1978). The radioactivity level of the DNA extracted from the silk gland was meastred in a toluene-triton scintillator with a packerd liqued scintillation counter. The activity of DNA synthesis was expressed as the level of radioactivity (dmp per pair of silk glands).

Juvenile hormone analogue

The juvenile hormone analogue (JH) used here was methoprene (ZR 515), generously supplied by Dr. Shimada, Ohtsuka Pharmaceutical Company, Tokushima. An acetone solution containing 1 μg of the drug per 5 μl of acetone was applied topically to the larvae for the first three days of the 5 th instar, once a day at a dose of 5 μl of solution per gram of live body weight.

Determination of tissue protein of the posterior silk gland

A pair of posterior silk glands was fixed with 5 % TCA for 2 hours and frozen at -20℃ overnight. The silk glands were scraped off with pincettes in 5 % TCA solution separated into tissue protein and fibroin. The tissue protein was homogenized in 5 % TCA solution and boiled for 15 min. After having been chilled in an ice bath, the tissue protein was precipitated by centrifugation at 3,000 rpm for 15 min. to remove nucleic acids and was then dried and weighed.

Results

DNA accumulation in the posterior silk gland

DNA accumulation in the posterior silk gland increased from the first day of the 5 th instar and reached a maximum on the 5 th day in the control larvae. When JH was applied to the larvae, the DNA accumulation was gradually increased for three days, after which it increased rapidly and exceeded that of the control larvae on the 7 th day (Fig. 1).

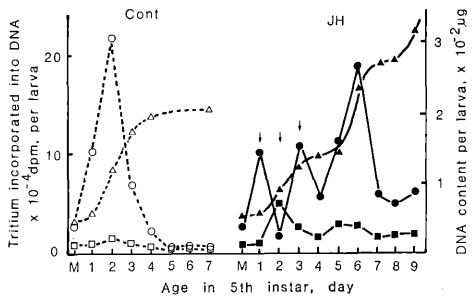

Fig. 1. Daily change in DNA content and DNA synthetic activity in the posterior silk glands of the 5 th instar larvae treated with JH. ○ △, □, Control larvae; ●, ▲, ■, JH treated larvae; △, ▲, DNA content; ○, ●, ^{3}H-TdR incroporated; □, ■, ^{3}H-dTTP incorporated. Arows show the periods when JH. was applied.

Effect of JH application on DNA synthetic activity

Levels of DNA synthetic activity in the silk gland are also shown in Fig. 1. A peak of [^{3}H]-TdR activity incorporated into the DNA was observed on the 2nd day of the 5 th instar and the activity disappeared after the 4th day of the 5 th instar. When JH was applied to the larvae the activity during the 5 th instar was markedly different from that of the control larvae ; the activity declined rapidly on the 2 nd day and recovered on the 3 rd day, approaching the control level, increasing again on the 6 th day.

The radioactivity level of [^{3}H]-dTTP incorporation into DNA reached a maximum on the 2 nd day and then decreased in the control larvae. In the JH-treated larvae, a peak of activity was observed on the 2 nd day and again on the 5 th day of the 5 th instar. The peak observed on the 2 nd day in the treated larvae was about two times higher than that in the control larvae. This phenomenon may have been due to a deficiency of the dTTP pool resulting from a decline of thymidine kinase activity due to JH application (Kurata and Daillie, 1978 ; Kurata, 1978).

Daily change in interacellular cyclic AMP and GMP concentration

Figure 2 shows the daily change in intracellular cyclic AMP concentrations in pairs of silk glands from larvae both with and without JH treatment, from the 4 th day of the 4 th instar to the end of the 5 th instar. In the control larvae, the concentration of cyclic AMP was 3 pmol on the 4 th day of the 4 th instar and rose rapidly after the beginning of the 5 th instar, reaching a maximum of 75 pmol on the 5 th day. When JH was applied to the larvae the concentration of cyclic AMP rose very slowly for two days after application and then rose, reaching a maximum of 118 pmol on the 12 th day of the 5 th instar.

The daily change in intracellular cyclic GMP concentration in the posterior silk glands is shown in Fig. 3. The cyclic GMP concentration was 0. 4 pmol on the 4 th day of the 4 th instar, rising rapidly during the 5 th instar to reach a maximum of 24 pmol on the 5 th day. When JH was applied to the larvae the concentration

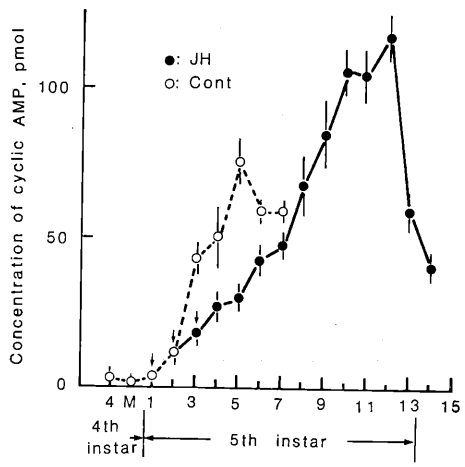

Fig. 2. Daily change in cyclic AMPin the posterior silk glands in the 5 th instar larvae treated with JH. ○, Control larvae ; ●, JH treated larvae.

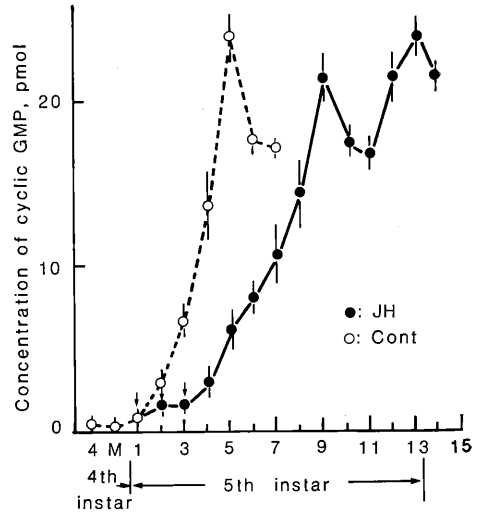

Fig. 3. Daily change in cyclic GMP in the posterior silk glands in the 5 th instar larvae treated with JH. ○, Control larvae ; ● JH treated larvae,

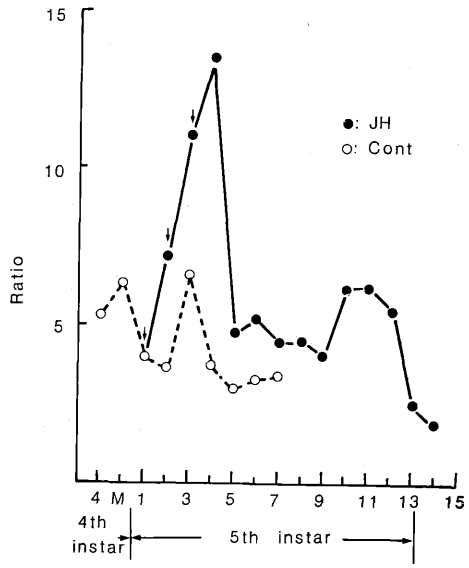

Fig. 4. Daily change in the ratio of cyclic AMP content to cyclic GMP content. ○, Control larvae; ●, JH treated larvae.

of cyclic GMP on the 2 nd day of the 5 th instar was 65% that of the control larvae and then increased and reached a first peak of 24 pmol on the 9 th day and a 2 nd peak of 25 pmol on the 13th day of the 5 th instar.

Alteration of the ratio of cyclic AMP tocyclic GMP

To clarify whether increases in cyclic AMP concentration paralleled increases in cyclic GMP concentration in the posterior silk gland, the quantitative ratio of cyclic AMP to cyclic GMP was estimated. There were periods when the increase in concentration of cyclic GMP was much reduced, and these appeared as peaks in the curve of the ratio (Fig. 4). In the control larvae, the first peak appeared on the 4 th molting, and the second peak on the 3 rd day of the 5 th instar. When JH was applied to the larvae the corresponding peak on the 3 rd day in the controls appeared on the 4 th day and an additional peak was observed on the 11 th day of the 5 th instar.

Content of tissue protein in a pair of posterior silk glands

The tissue protein content of the posterior silk gland was 38 mg on the 5 th day in the control larvae and 50. 5 mg on the 12th day in the JH-treated larvae. Periods of tissue protein measurement corresponded to the times when the content of each cyclic nucleotide reached a maximum level in the posterior silk gland.

Discussion

As shown in the results, the maximum concentrations of intracellular cyclic AMP in the posterior silk glands were 75 pmol and 24 pmol, respectively and the ratio of cyclic AMP to cyclic GMP was 3. 1 in the control larvae. Goldberg *et al.* (1973) have shown that the quantitative ratio of cyclic AMP to cyclic GMP was 10-100 in mammalian tissues. Although Ishikawa *et al.* (1969) reported that crickets contained a high level of cyclic GMP, which was 2-3 times higher than the content of cyclic AMP, Fallon and Wyatt (1975) demonstrated that the ratio in tissues from the criket, *Acheta domestica* (L) varied according to the tissue and the content of cyclic GMP was not higher than that of cyclic AMP in all tissues examined.

The posterior silk glands of the silkworms used in this study contained 2 pmol of cyclic AMP and 0. 6 pmol of cyclic GMP per mg of tissue protein in a pair of posterior silk glands. Each concentration corresponded to that of the tissue having a low concentration of each nucleotide in the crikets reported by Fallon and Wyatt (1975).

There have been many studies on the interaction between change in the concentration of cyclic nucleotides and the initiation of DNA synthesis in mammalian tissues. These studies have shown that a brief rise in cellular cyclic AMP content is necessary for the initiation of DNA synthesis in T51B rat liver epitheloid cells (Boynton and Whitfield, 1979), in regenerating hepatocytes (Br ϕ nstad and Christoffersen,

1981) and primary cultures of adult rat hepatocytes (McGowan *et al.*, 1981). Intraperitoneal injection the β-adrenergic agonist *dl*-isoprotenol hydrochloride into rats caused an early very large cyclic AMP surge in the parotid gland, followed by second surge which induced DNA synthesis (Tsang *et al.*, 1980). Furthermore, the addition of an increasing concentration of fibroblast growth factor in the presence of hydrocortisone caused concomitant increase in both cyclic GMP concentration and the eventual induction of DNA synthesis and cell division (Rudland *et al.*, 1974).

In the posterior silk gland studied, the increaes in cyclic AMP and GMP levels parallelled an increase in DNA synthetic activity and then ceased with the disappearence of DNA synthetic activity. Although three peaks in DNA synthetic activity were induced by administration of JH to the larvae, only one peak in each cyclic nucleotide level occurred in the posterior silk gland during the period of DNA synthesis(from the lst to the 9 th day of the 5 th instar). Therefore, the effect of inducing DNA synthesis by an increase in interacellular cyclic nucleotides similar to that found in tissue-cultured cells of mammals was not observed in the posterior silk gland of the silkworm.

The quantitative ratio of cyclic AMP to cyclic GMP rose at the 4 th molting and on the 3 rd day of the 5 th instar in the control larvae, and on the 4 th day and the 10th day of the 5 th instar larvae treated with JH. The activity of DNA synthesis increased following the peak at the 4 th molting in the control larvae and the peak on the 4 th day of the 5 th instar in the JH-treated larvae, but was not observed following the peak on the 3 rd day of the 5 th instar in the control larvae and the peak on the 11th day of the 5 th instar in the JH-treated larvae.

To clarify the interaction between the initiation of DNA synthesis and the surge in the ratio of cyclic AMP to cyclic GMP, we need more extensive data on the concentration of cyclic nucleotides at an early stage of silkworm development.

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倉田啓而：家蚕糸腺のサイクリック AMP および GMP 量の変化と DNA 合成

絹糸腺のサイクリック AMP および GMP 量の変化と DNA 合成活性の変化を幼若ホルモン投与蚕および非投与蚕について測定した。サイクリック AMP および GMP 量は5齢初期より次第に増加し，それぞれの最高値は投与蚕では 118 pmol および 25 pmol，非投与蚕では 75 pmol および 24 pmol であった。最高値時のサイクリック AMP および GMP の組織たんぱく質量（mg）当の量は投与蚕では 2.3 pmol および 0.5 pmol，非投与蚕では 2 pmol および 0.6 pmol であった。サイクリック AMP および GMP 量の増加は DNA 合成活性と符合し，DNA 合成活性が消失するとそれは低下した。またこれら2サイクリックヌクレオチド量の増加と DNA 合成開始について考察を加えた。