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Change in nuclear potassium electrochemical activity and puffing of potassium-sensitive salivary chromosome regions during *Chironomus* development

(K⁺-sensitive microelectrode/*Chironomus tentans*/oligopause/flameless atomic absorption spectrometry)

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ABSTRACT **Changes in nuclear K^+ electrochemical activity and total nuclear K^+ content in salivary glands of *Chironomus tentans* were measured with ion-selective microelectrodes based on valinomycin and with flameless atomic absorption spectrometry, respectively. The K^+ activity increased by a factor of 2.6 and the total K^+, by a factor of 1.5 as oligopausing larvae developed into prepupae. The extent of decondensation (puffing) of K^+-sensitive regions in the polytene chromosomes underwent a parallel increase during this developmental event. *In vitro* culture of glands from oligopausing larvae resulted in similar changes with respect to nuclear K^+ activity and puffing.**

Changes in nuclear ion concentrations have been suggested to be involved in fundamental biological processes such as egg maturation and activation, cell replication and differentiation, aging, regeneration, and hormone action (1–7). Moreover, many constituents of nuclei (including chromatin and RNA and DNA polymerases) exhibit specific ion sensitivities (for references see refs. 5 and 8). Unfortunately, data (5, 9–11) on *in vivo* ion changes within cell nuclei are scarce and of limited utility, because the methods used (atomic emission and absorption spectrometry and neutron or electron activated γ- and x-ray emission analysis) permit the measurement of the *total contents* of the particular ions only—i.e., of the sums of their bound and free fractions. However, the ion sensitivities of nuclear constitutents most likely depend above all upon the free fractions of ions, more specifically on their *electrochemical activities*.

In order to be able to determine ion activities directly, we decided to use ion-sensitive microelectrodes. Here we report results obtained with a K^+-sensitive microelectrode based on the neutral ionophore valinomycin (12, 13). The larval salivary glands of *Chironomus* are especially suitable for both electrophysiological and cytological analyses because of their giant nuclei and chromosomes.

Two stages of fourth instar larvae of *C. tentans* with extremely different metabolic, transcriptive, and morphogenetic activities were compared: (*i*) *oligopause*, a form of dormancy (14) in which many chironomids hibernate (15), and (*ii*) the *prepupal* (pharate pupal) *stage*, characterized by dramatic differentiation processes in connection with metamorphosis.

MATERIALS AND METHODS

Double-barreled microelectrodes (overall tip diameter of approximately 1.5 μm) were prepared by a method described by Oehme and Simon (12) with a few modifications. Instead of θ capillaries, we used double pyrex tubings with an inner filament from Clark Electromedical Instruments, Pangbourne, England. The ion-selective component was 5% valinomycin/2% potassium tetra-*p*-chlorophenylborate/25% 2,3-dimethylnitrobenzene dissolved in dibutylsebacate. (The nitrobenzene was added to reduce the electrode's resistance.) The reference barrel was backfilled with 2 M lithium acetate. Such K^+-sensitive microelectrodes had an average slope of 57.6 ± 0.7 mV (SEM) per decade activity change in the range of 1 to 200 mM KCl and a very high selectivity for K^+ versus other commonly found cations as well as versus lipophilic ammonium ions such as acetylcholine. The following selectivity factors (16) were obtained by the separate solution method (17) in 0.1 M cation chloride solutions: $\log K^{Pot}_{K,Li}$, −4; $\log K^{Pot}_{K,Na}$, −3.2; $\log K^{Pot}_{K,Mg}$, −5.0; $\log K^{Pot}_{K,Ca}$, −4.5; $\log K^{Pot}_{K,acetylcholine}$, −2.5. These parameters correspond very well with those of valinomycin-based macroelectrodes used in flow-through systems (18) and commercial blood analyzers (19). The inconvenience of a relatively high tip resistance (approximately 10^{11} Ω) and, thus, of a rather long response time (time for a 10–90% change of the eventual signal, normally 30 sec) is compensated by the otherwise excellent performance of the valinomycin-based microelectrode, in terms of selectivity and slope. The tip potential of the reference barrel changed by less than 3 mV when the external bathing solution was switched from an "extracellular" (100 mM NaCl/3 mM KCl) to an "intracellular" (20 mM NaCl/100 mM KCl) ion composition, measured against a 3 M KCl/agar bridge. [The activities of all salt solutions were calculated according to Bates and Alfenaar (20).] The drift of the electrodes was less than 2 mV as was routinely determined by calibration before and after each intranuclear measurement. Both the a_K signal and the reference signal (i.e., the membrane potential) were monitored with high-impedance buffer amplifiers [type 3431 K, from Burr Brown Res. Corp. (Tuscon, AZ), input resistance of 10^{14} Ω, input capacitance of 2 pF].

For intranuclear measurements of the K^+ activity (a_K), salivary glands were explanted and mounted in an insect tissue culture medium (21). The double-barreled electrode was implanted with the aid of a micromanipulator. The exact intracellular position of its tip was monitored under an inverted microscope (40× objective, 16× eyepieces) with a depth of focus of less than 4 μm (note that the diameter of a nucleus is more than 50 μm). Constant a_k readings were obtained 2 min after the electrode's implantation. Thereupon, the readings stayed constant for at least 10 min (exceptions are discussed in the following section).

The total K^+ content was determined by flameless atomic absorption spectrometry of nuclei isolated in the lyophilized state. The salivary glands were frozen (and subsequently lyophilized) *in situ:* the glands and the nuclei were manually isolated under silicone oil, which does not take up any measurable amounts of water, Na^+, or K^+; the nuclear dry weight was determined on an evacuated quartz fiber balance (22).

Oligopause in the middle of fourth instar of *C. tentans* was

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induced by a short-day regimen (6 hr light/18 hr dark), whereas synchronous development towards prepupa was started by a switch to long-day conditions (18 hr light/6 hr dark). Additional care was taken to obtain as homogeneous a population of animals as possible within the given stages by applying the modified culturing and staging methods originally outlined in refs. 15 and 23; a detailed description of the method will be given elsewhere.

For chromosomal analyses, the experimental gland and its unimpaled sister gland were processed into orcein/acetic acid-stained squash preparations after the a_K measurement or immediately after decapitation of the animal, respectively. The extent of decondensation (puffing) in specific regions of the polytene chromosomes was determined by a described method (24).

RESULTS AND DISCUSSION

The K^+ electrochemical activities of the nuclear sap of salivary gland cells of oligopausing larvae and prepupae are given in Table 1. The potential differences across the cell membrane were determined to be -3.1 ± 0.61 mV and -16.3 ± 3.23 mV (mean ± SEM) with these two stages. No significant differences between nuclear and cytoplasmic a_K or potential difference were found (10 experiments), confirming previous findings by Palmer and Civan (25). However, these authors found higher a_K and more negative potential difference values than we did, but the two sets of data are difficult to compare for the following four reasons: Palmer and Civan used (*i*) another species of *Chironomus* (i.e., *C. attenuatus*), (*ii*) another stage (i.e., late fourth instar), and (*iii*) a highly hypertonic medium (i.e., Ringer solution of 293 mosM.). We determined the osmolarity of the hemolymph and of our medium to be 200 mosM and 215 mosM, respectively. (*iv*) Moreover, they used Corning ion exchanger solution (no. 477317) as the potassium-selective component, which responds 300 times better to acetylcholine than to K^+ (12). Because the intracellular activities of acetylcholine and other lipophilic ammonium ions are unknown at the present, a considerable uncertainty is introduced by using Corning's ion exchanger for a_K measurements.

Our results in Table 1 demonstrate that in the course of development from oligopause to prepupal stage the intranuclear K^+ activity increased by a factor of 2.6. In accordance with the results of Kroeger and coworkers (9), the total K^+ content of nuclei also increased, but only by a factor of 1.5 (Table 1). This could be explained by the existence of a rather large fraction of bound K^+, which remains more or less constant during development. The same may be concluded from a transformation of the data given in Table 1 into hypothetical concentrations. If one assumes that the ion activity coefficient in the nucleus is the same as in the incubation medium (i.e., 0.76) and that the nuclear water content is 80% (9), the concentrations of total K^+ are always higher than those of free K^+ by 30–60%. The existence of bound nuclear K^+ would be an interesting and new finding. However, it has to be reemphasized that the above concentrations are only estimates, because no exact figures for the single ion activity coefficient of K^+ and the water content in the nuclei investigated are available at the present time.

During the same developmental period (i.e., from oligopause to the prepupal stage), the general transcriptional and puffing activity in the salivary gland nuclei also increases (26). As is indicated by the measurements referred to in Table 1, chromosome region I-18-C drastically expands and decondenses. The same holds for regions IV-2-B and BR 1 (26). All three chromosome regions have been demonstrated with isolated nuclei or chromosomes to be responsive to an increase of K^+ in their environment (27–30) and, with whole animals, to react to an *in vivo* ecdysone treatment (31, 32). It has to be emphasized that the measured changes of the free and of total K^+ concentration during development (approximately 50 mM and 40 mM, respectively, same assumptions as above) are larger than those previously found to be necessary for an induction of the above chromosome decondensation (i.e., 35 mM) in isolated nuclei (27, 28).

For calculation of the a_K values in Table 1, only readings of the first impalement of each gland were used. With either developmental stage, these readings were constant for more than 10 min. However, as evident from the data given in Table 1 for oligopause, the nuclear a_K increased considerably within 1 min when the electrode was withdrawn from the first nucleus impaled and was reimplanted into the nucleus of a second cell of the same gland. Interestingly enough, this treatment caused the K^+-sensitive chromosome regions I-18-C (values given in Table 1), IV-2-B, and BR 1 to expand, which was determined with the same glands previously used for the a_K measurements; their total *in vitro* incubation time (= time between explantation and fixation) amounted to approximately 30 min. At present, the reason for this rapid effect of withdrawal and reimplantation of the electrode is a matter of speculation. It might be related to an experimentally induced alteration of the cell membrane's function, which is concluded from the observation that, with the second impalement (in contrast to the first one), the cell membrane's potential temporarily broke down or even became positive but recovered afterwards over an extended period of time. The susceptibility of glands to respond to a repeated ex-

Table 1. Developmental and experimentally induced changes of total K^+ and K^+ activity (a_K) in salivary gland nuclei; comparison with expansion of chromosome region I-18-C

Developmental stage*	Total K^+,† mg/g dry weight	a_K of first impalement,† mM	Change in a_K upon 2nd impalement,‡ mM	Expansion of chromosome region I-18-C†§	Change of chromosome region I-18-C in impaled gland¶
Oligopause	12 ± 2 [5;34]	25 ± 10 [14;14]	+28.8 $P < 0.001$ [9]	1.32 ± 0.17 [14;112]	+0.37 $P < 0.001$ [9]
Prepupa	18 ± 3 [5;35]	64 ± 15 [17;17]	+1.18 $P = 0.75$ [13]	2.16 ± 0.36 [12;96]	−0.22 $P = 0.1$ [10]

In brackets are numbers of animals and nuclei.

* Stage 4.5 for oligopause and stage 10 for prepupa, according to the classification by H. Ineichen (unpublished results) based on the status of the imaginal discs (23).

† Mean ± 99% confidence limits.

‡ Difference between the mean a_K readings of the second and the first impalement of each gland; probability P for difference being zero (Student's t test).

§ Determined in the immediately fixed unimpaled sister gland according to ref. 24; arbitrary units.

¶ Differences between the mean expansion in the experimental gland (fixed at the end of the a_K measurement) and its immediately fixed unimpaled sister gland; P same as in ‡.

perimental puncturing by a transient depolarization and subsequent hyperpolarization of the cell membrane, as well as by an increase in a_K and expansion of chromosome region I-18-C in their nuclei, appears to be stage-specific, because it could not be observed with salivary glands of prepupae under identical experimental conditions (Table 1).

Because the a_K may change under certain *in vitro* conditions, the question arises whether the observed developmental stage-specific difference in nuclear K^+ activity really represents the *in vivo* situation or just reflects a differential sensitivity of the glands of different stages towards *in vitro* treatment. Since it was realized that wounding is indeed a problem in using explanted tissues, the parallel measurements of total nuclear K^+ were performed, which are based on a method that avoids the isolation of fresh glands. The results of these measurements strongly support those obtained with the K^+-sensitive electrode in terms of development-specific differences in nuclear K^+.

The present results demonstrate that the K^+ activity does change in the cell nucleus, both during certain developmental periods as well as after repeated impalement of the tissue. The parallel induction of decondensation in chromosome regions that had been shown with isolated nuclei and chromosomes (27–30) to be responsive to an increase of K^+ in their environment is compatible with the so-called ion hypothesis of gene regulation (7, 33). Preliminary results (R. Mähr, personal communication) indicate that the changes of nuclear K^+ within the concentration ranges we have measured produce significant effects on RNA synthesis in mass-isolated salivary gland nuclei of *Chironomus* (34).

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