Influence of methotrexate on respiration and ultrastructure of meristematic cells from *Allium cepa* L. roots

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Abstract

It has been demonstrated that methotrexate (an oncostatic preparation) inhibits cellular respiration and reduces the permeability of plasmalemma and tonoplast in cells of *Allium cepa* roots. Changes were also found in the ultrastructure of meristematic cells, consisting presence of numerous mitochondria with a dense matrix, a highly developed system of cristae and an increased number of vacuoles frequently derived from the ER.

INTRODUCTION

Methotrexate, an oncostatic preparation belonging to the antimetabolites of folic acid (Boesen and Davis 1969) exhibits typical cytostatic effects, mainly by inhibition of DNA synthesis. This has been demonstrated both *in vivo* and *in vitro* (Skeel et al. 1976, Skoog et al. 1976).

MATERIAL AND METHODS

The experiments were performed with adventitious roots of *Allium cepa* L. For methods of culture, fixation and staining see Podbielkowska (1981).

Respiration was tested on control roots and those incubated for 24 h in 10^{-5} M methotrexate in Warburg apparatus (Dixon 1951). Uptake of O_2 /ml/g fresh weight was measured in 1-cm root apices for 2 h at 10-min intervals. Seven experimental series were run with 4 samples in each.

For establishing the permeability of the plasmalemma and tonoplast, fragments of the epidermis of the upper bulb scale were incubated for 60 min in 10^{-5} M methotrexate solution and then plasmolysed in 30 per cent sucrose solution. Deplasmolysis time was studied in 0.6 M ethylene glycol solution which permeates rather slowly into the cell. Time was measured from the moment of placing the tissue in the solution of the deplasmolyser to the moment of complete deplasmolysis of about 20 cells in ten replications.

RESULTS

The protoplast structure in cells of the protoparenchyma lying above the initial layer (zone) was analysed in a light and electron microscope. The root growth apices treated with methotrexate were compared with control material (Figs. 8, 9).

It was found that, in meristematic cells treated with the cytostatic, numerous, sometimes swollen, mitochondria appear with well developed cristae and an electron-dense matrix (Figs. 1, 2, 3) as well as dictyosomes with frequently characteristically enlarged cisternae (Fig. 1). The endoplasmic reticulum morphologically similar to that of the control gives often rise to vacuoles (Fig. 1) or undergoes fragmentation producing vesicular forms. Vesicles or ER cisternae of various length were also observed in the regions between the cell wall and the plasmalemma detached from it (Figs. 5, 6, 7). In these regions and also in the cytoplasm sporadic myelin structures were noted (Fig. 4).

It results from oxygen uptake measurements that after 24 h of exposure to the action of methotrexate the cellular respiration level dropped on the average by 17 per cent, that is to 83 per cent of that of the control (Table 1). This effect remained stable for the 2-h period of duration of the experiment for each of the seven experimental series.

Table 1
Influence of methotrexate on the respiration of root growth apices
of *Allium cepa*

Successive experimental series	% as compared with control (mean values)
1	76.8
2	79.3
3	88.7
4	95.3
5	71.0
6	81.1
7	89.7
Mean	83.1

Figs. 1, 2. Meristematic cells of root growth apices from *Allium cepa* treated with methotrexate for 12 h

Numerous polymorphic mitochondria and dictyosomes with changed structure, 2% KMnO_4 , $\times 20\,000$

Figs. 3, 4, 5. Meristematic cells of root growth apices from *Allium cepa* treated with methotrexate for 12 h

Fig. 3. — mitochondria with dense matrix and modified cristae; Fig. 4 — myelin structure close to cell wall; Fig. 5 — numerous vesicles and modified ER cisternae close to cell wall. GA after Karnovsky, $\times 24\,000$

Figs. 6, 7. Meristematic cells of root growth apices from *Allium cepa* treated with methotrexate for 12 h. Characteristic detachment of plasmalemma from cell wall and accumulation in aplasmatic areas of vesicles with dense contents as well as of other structures resembling degraded cytoplasm. GA after Karnovsky, $\times 35\,000$

Figs. 8, 9. Meristematic control cells of root growth apices from *Allium cepa*
 Fig. 8 — 2% KMnO_4 , $\times 20\,000$; Fig. 9 — GA after Karnovsky, $\times 35\,000$

Plasmalemma and tonoplast permeability were tested by the plasmolytic method. It results from the data in Table 2 that methotrexate reduces this permeability.

Table 2

Influence of methotrexate on the permeability of the plasmalemma and tonoplast in epidermal cells of the bulb scale

Duration of deplasmolysis (min)	Deplasmolysed cells in successive minutes of experiment (%)	
	methotrexate	distilled water
3	0	5
6	5	10
9	10	20
12	25	60
15	45	100
18	75	—
21	100	—

DISCUSSION

Methotrexate is one of the most commonly applied drugs of the antimetabolite group (Boesen and Davis 1969). As most cytostatics it is highly toxic.

The results of the present experiments demonstrated that methotrexate only slightly reduces cellular respiration and membrane permeability. This is associated with minor changes in the ultrastructure of protoplast. As most characteristic changes were considered: the presence of numerous polymorphic mitochondria with electron-dense matrix and a well developed system of cristae. These features of the ultrastructure rather seem to indicate intensive respiratory metabolism. Swelling of the mitochondria associated with states of energetic deficit was less frequently noted. Certain changes were also observed in the morphology of the endoplasmic reticulum: vacuolisation and also fragmentation of cisternae migrating in the form of segments of various length in the regions of plasmalemma characteristically detached from the cell wall. Cytoplasmic membranes play a role both in respiratory processes (glycolysis) and in the regulation of active transport. ATP arising during electron transport in the microsomal respiratory chain localised within the ER membranes is utilised here.

The sporadic myelin structures observed by us within the cytoplasm and in aplasmatic areas close to the cell wall may have arisen due to

the toxic action of the oncostatic. The myelin structures are, namely, considered by some authors as a certain form of degradation of ER or of mitochondrial membranes, caused by the action of toxins.

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Wpływ metotreksatu na oddychanie i ultrastrukturę komórek merystematycznych korzeni Allium

Streszczenie

Badano oddychanie komórkowe, przepuszczalność błon oraz zmiany w ultrastrukturze komórek poddanych działaniu metotreksatu — preparatu onkostatycznego z grupy antymetabolitów. Metotreksat powodował inhibicję procesów oddechowych oraz ograniczał przepuszczalność błon. Efekty te skorelowane były ze zmianami w ultrastrukturze tkanki merystematycznej. Wyrażało się to obecnością licznych mitochondriów, niekiedy napęczniałych, z rozwiniętym systemem krist i elektronowo gęstą matrix oraz obecnością diktiosomów o zmienionej strukturze. Obserwowano także grubienie ściany komórkowej.