Ionic relations and electrophysiology of single cells of *Characeae*

Part II. The effect of IAA on sodium and potassium influx in cells of *Nitella translucens*

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INTRODUCTION

It has been shown that IAA (β-indolylacetic acid) has a stimulatory effect on potassium and rubidium uptake by plant cells (Commoner and Mazia 1962; Higinbotham et al. 1962). According to Pickles and Sutcliffe (1955) IAA inhibited potassium efflux from red beet tissue and increased sodium influx. These data are of interest here because both fluxes seemed to be passive, which follows from Higinbotham's (1960) consideration of electrochemical potential gradients in cells of higher plants. Ilan (1962) demonstrated a stimulatory effect of IAA on potassium uptake by hypocotyl tissue of sunflower associated with a simultaneous inhibition of ammonium influx. Ilan and Reinhold (1962) suggested that IAA affects the molecules of ion carriers across membranes in such a way that their affinity for potassium increases. This conclusion is based on Handley and Overstreet's hypothesis (1961) that $K^+$, $Rb^+$, $NH_4^+$ compete for the sites on a carrier molecule transporting these ions across the plasma membrane.

Ilan and Reinhold (1963) have shown that IAA stimulate potassium uptake into “osmotic volume” in cells of sunflower hypocotyl. The addition of sucrose to the medium (10^{-2} M) stopped the stimulatory effect of IAA on K^+ uptake. It is worthwhile mentioning that the herbicide 2,4-D at a concentration of 2·10^{-4} M causes potassium uptake in higher plants to decrease (Swenson and Bonner 1960). According to Hansson and Bonner (1954) the addition of 2,4-D leads to an increase in the exchange capacity of hypocotyl tissues. This fact might indicate that IAA stimulated potassium uptake into the “Donnan free space”, located in the cell wall.

So far the effect of IAA on ionic relations in single internodal cells of *Characeae* has not been studied. Meanwhile these cells seem to constitute an excellent material for such investigations and it is possible to correlate these investigations with electrophysiological measurements. It was shown
(Stolarek 1966) that IAA changes substantially electric parameters of single cells of Characeae. In the present work an attempt of a closer analysis of the effect of IAA on the permeability of plant cells was made.

MATERIAL AND METHODS

The measurements of sodium $M^+_Na$ and potassium $M^+_K$ influx were made in internodal cells of Nitella translucens collected in Dunkeld, Scotland. Prior to the measurements the cells were stored in the solution known as APW (NaCl 1.0 mM, KCl 0.1 mM and CaCl$_2$ 0.1 mM). The cells used for the experiments had a length of 5—10 cm.

Sodium influx was measured with the use of $^{22}$Na which was added to the medium as $^{22}$NaCl substituting part of the sodium chloride in the medium. Specific activity of such a medium was 5—10 $\mu$C/g equiv. of Na. Cells of known length and diameter were placed in radioactive medium for 6 hours. After loading the cells were taken out, washed out with APW for 20 min. and blotted with filter paper. One end of the cell was cut off and the cell sap was collected with “microcup” micropipettes. The sample of cell sap amounting to 5—30 $\mu$l was transferred to a container and diluted to a volume of 10 ml.

The radioactivity was measured with a scintillation counter “Ecco N 664A” joined to an automatic scaler “Ecco N 610A”. The background was 1.5 to 2 imp./sec. The activity of the cell sap was compared with that of 0.1 ml of labelled APW and diluted to a volume of 10 ml prepared at the moment when the cells were put in to the radioactive solution.

Potassium influx was measured with $^{42}$K. This isotope was received as $^{42}$K$_2$CO$_3$ and transferred into potassium chloride by titration with hydrochloric acid. The labelled potassium chloride was then substituted for part of the potassium chloride in the medium in such a way as to obtain specific activity of the solution of 1 $\mu$C/ml. In experiments with radioactive potassium like those of Hope and Walker (1961) the results of the measurements of the radioactivity with two methods were compared. The first method of obtaining samples has just been described and the other consisted in measuring radioactivity of intact cells. The accuracy of the second method depends on the degree of removal of the radioactivity from the cell wall. Both methods gave similar results i.e. potassium influx measured with both methods did not differ substantially. Potassium influx was measured with a Geiger-Müller M6 20th Century Electronics counter.

In the case of potassium influx measurements of the radioactivity of sap samples or intact cells were compared with the radioactivity of small amount of the medium prepared at the beginning of the experiment.

The net uptake of ions in a cell can be written in the usual form:

$$\frac{dC^*_i}{dt} = M^i \frac{A}{V} \frac{C^*_o}{C_o}$$
where \( V \) and \( A \) are volume and surface area of the cell respectively, \( M' \) is the influx. The radioactivity \( C_i^* \) of the cell was determined after a given period of loading and since other parameters were known, the influx \( M \) was calculated.

RESULTS

Sodium influx

Sodium influx was measured with the \(^{22}\text{Na} \) isotope. The cells were soaked in radioactive solution for 6—10 hours and then the cell sap was taken. The control results were obtained on 24 cells soaked in a solution of the composition: 0.1 mM KCl, 0.1 mM CaCl\(_2\) and 1.0 mM NaCl referred to as APW. The mean value of sodium influx was 0.418 pM/cm\(^2\) \cdot sec \pm 0.062. In another series of experiments performed on 10 cells the effect of IAA (2\( \times 10^{-5} \) M) was investigated. IAA was added to APW two hours before the experiments started. pH value was adjusted to 7.2 with phosphate buffer. The average value of sodium influx in this case was 0.467 pM/cm\(^2\) \cdot sec. i.e. was close to the control.

Potassium influx

a) Control conditions. The experiments were performed in a room with natural diurnal light and a luminescent illumination in the evening and night. The cells soaked in radioactive solution were taken out at intervals of 2 hours and their radioactivity was measured. Before and during the experiments the cells were soaked in APW. Potassium influx calculated as a mean for 39 cells is 0.732 pM/cm\(^2\) sec. (Table 1, row 1).

Potassium uptake as a function of time is shown in Fig. 1. It is worth mentioning that \(^{42}\text{K} \) uptake is linear during a long period, which is in agreement with MacRobbie’s (1962) data.

b) Effect of IAA on potassium uptake. Two hours before the beginning of the experiment the cells were placed in the following solutions: APW + + IAA(2 \( \times 10^{-5} \) M), APW + IAA(2 \( \times 10^{-4} \) M). The pH of IAA solutions was adjusted to 7.2 by adding small quantities of 1/100 N NaOH. Concentration of sodium ions did not change significantly. If there is any effect of rising sodium concentration on potassium influx it might only be a decrease of potassium influx, which may be neglected owing to the large stimulatory effect of IAA.

The results obtained on 58 cells treated with 2\( \times 10^{-5} \) M IAA and 15 cells treated with 2\( \times 10^{-4} \) M IAA are gathered in Table 1. It is to be seen that in the first case influx was stimulated (1.224) as compared to the control (0.732), while in the second case potassium uptake was considerably decreased (0.221).
A number of observations were made on the effect of IAA on the growth of Characeae cells showing that auxin at a concentration of $2.10^{-5}$ M stimulated cell growth while that of $2\times10^{-4}$ M inhibited it.

c) **Effect of ouabain on potassium uptake** by cells pretreated with IAA. Ouabain (G-strophanthin) is known as an inhibitor of active potassium transport in plant and animal cells. According to MacRobbie (1962), ouabain does not affect sodium fluxes in *Nitella translucens* but it reduces active potassium influx. The effect of ouabain on potassium uptake in control cells and those pretreated with IAA 2 hours before the experiment was investigated (Table 1, rows 4 and 7; Fig. 1).

d) **Effect of Mg$^{2+}$ on potassium uptake** in cells pretreated with IAA. From the last section it appears that ouabain inhibits potassium uptake, and evidently the active part of the influx is in question (MacRobbie 1962). Gonda and Quaestel (1962) suggested that the mechanism of ouabain effect consists in blocking the potassium carrier across cell membrane. According to Skou (1963), ouabain inhibits the formation of ATPase. ATP•Mg$_2$ complexes playing an essential role in the active potassium transport mechanism. In the present work the effect of magnesium ions on potassium uptake was investigated. Mg$^{2+}$ ions were used as MgCl$_2$ at a concentration of 5 mM. An increase in potassium influx was observed in cells bathed by the external solution containing APW+IAA($2\times10^{-5}$ M)+Mg$^{2+}$ as compared to the cells soaked in APW+IAA without
Table 1
Potassium influx in cells of *Nitella translucens* in various conditions
pM·cm⁻²·sec⁻¹

<table>
<thead>
<tr>
<th>External medium</th>
<th>Number of cells</th>
<th>Influx (± s.e.m.)</th>
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<tr>
<td>1 APW (CONTROL)</td>
<td>39</td>
<td>0.732±0.018</td>
</tr>
<tr>
<td>2 APW+IAA (2·10⁻⁵ M)</td>
<td>70</td>
<td>1.224±0.047</td>
</tr>
<tr>
<td>3 APW+IAA (2·10⁻⁴ M)</td>
<td>15</td>
<td>0.226±0.037</td>
</tr>
<tr>
<td>4 APW+ouabain (5·10⁻⁵ M)</td>
<td>16</td>
<td>0.419±0.041</td>
</tr>
<tr>
<td>5 APW+IAA (2·10⁻⁵ M)+ ouabain (5·10⁻⁵ M)</td>
<td>20</td>
<td>0.727±0.065</td>
</tr>
<tr>
<td>6 APW+Mg²⁺(5 mM)</td>
<td>10</td>
<td>0.654±0.064</td>
</tr>
<tr>
<td>7 APW+IAA (2·10⁻⁵ M)+ ouabain (5·10⁻⁵ M)+Mg²⁺(5 mM)</td>
<td>10</td>
<td>0.950±0.081</td>
</tr>
<tr>
<td>8 APW+DNP (10⁻⁵ M)</td>
<td>11</td>
<td>0.210±0.052</td>
</tr>
<tr>
<td>9 APW+2.4 D (10⁻⁵ M)</td>
<td>24</td>
<td>0.630±0.039</td>
</tr>
<tr>
<td>10 APW+IAA (2·10⁻⁵ M)+2.4 D (2·10⁻⁵ M)</td>
<td>10</td>
<td>0.222±0.137</td>
</tr>
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magnesium (Table 1, rows 5 and 7). The results of another experiment on cells remaining in APW+Mg²⁺(5 mM) showed no effect of magnesium on potassium uptake. Magnesium ions brought about a partial suppression of the effect of ouabain in the presence of IAA in the external medium.

e) *Effect of 2,4DNP and 2,4D on potassium uptake.* Dinitrophenol (2,4DNP) known as a phosphorylation decoupler applied at a concentration of 2·10⁻⁵ M reduced potassium influx by 75% (Table 1, row 8) whereas 2,4D (dichlorphenoxyacetic acid) did not have any effect on potassium influx. A rather peculiar result was obtained when IAA and 2,4D were added together to the external solution. Potassium influx diminished to 10% of the control value (Table 1, rows 10 and 1).

DISCUSSION

The measurements of sodium and potassium accumulation in cells of *Nitella translucens* allow to determine the permeability of the cytoplasm for these substances. The average control values of sodium and potassium influxes determined in the course of this work are close to the values given by MacRobbie (1962). It appears from the present work that IAA at a concentration of 2·10⁻⁵ M stimulates potassium uptake. The same
concentration of IAA was shown to stimulate growth of both higher plants and Characeae cells. The results of experiments on the effect of ouabain on potassium uptake in cells of Nitella translucens suggested that IAA induced an increase in both passive and active potassium influx (Table 2). The effect of magnesium on potassium accumulation in cells treated with IAA and ouabain could be related to the stimulation of transport ATP-ase activity by magnesium ions. The addition of magnesium ions to the control medium (APW) did not affect potassium uptake whereas it diminished inhibitory effect of ouabain.

<table>
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<td>Effect of auxin (IAA) on passive and active potassium influx in cells of Nitella translucens (pM.cm⁻², sec⁻¹)</td>
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| 1. Control influx | 0,732 |
| 2. Active part of the influx (component of the influx inhibited by ouabain) | 0,313 |
| 3. Passive part of the influx (1−2) | 0,419 |
| 1. Influx in the presence of IAA | 1,224 |
| 2. Active part of the influx (reduced by ouabain when auxin was present in the medium) | 0,500 |
| 3. Passive part of the influx (1−2) | 0,724 |

Gonda and Quastel (1962) proposed the following mechanism for the ouabain effect on active transport of potassium ions.

\[ X + ATP \rightarrow X - ATP \rightarrow XP_n + ADP \]
\[ X + ouabain \rightarrow X - ouabain \]
\[ X - P_n + K^+ \rightarrow K + X - P_n \rightarrow X + P_n + K^+ \]

where \( X \) is a carrier molecule of potassium across the cell membrane. The above written reactions express the effect of ouabain on the transport of adenosine triphosphatase and on active potassium transport. They also explain the antagonism between ouabain and potassium ions as far as their effect on adenosine triphosphatase activity is concerned (Dunnham and Glynn 1961). Skou (1963) suggested that ouabain blocks the formation of the complex ATP-ase.ATP.Mg₂. This complex plays an essential rôle in the mechanism of active potassium transport postulated by him. He suggested that the ATP molecule in the membrane can bind two magnesium atoms, one of which links ATP phosphates with the molecules of the protein belonging to ATP-ase.

According to A. Szent-Györgyi (1960) magnesium ions joined to the ATP molecule create a bridge between the electron system of ATP and that of the membrane. Binding of two Mg ions to ATP molecule causes weakening of the bond between ADP and P, and this eventually leads to the decomposition of the complex. According to Skou the binding between the molecules of ATP on the inner side of the membrane, and the site with a higher affinity for potassium than for sodium located
on the outer side of the membrane may arise owing to the electron shift induced by the process of joining magnesium ions to ATP molecules.

The observed phenomenon of the suppression of active potassium transport by ouabain can be interpreted as follows: if ouabain blocks the complexes of ATP-ase:ATP:Mg₂ then the addition of magnesium ions brings about the formation of an additional amount of these complexes, which can restore active potassium transport. However, magnesium ions added to the control medium or to the medium with ouabain only does not affect potassium uptake. It is possible that in this case the newly formed complexes are "inactive". Ouabain may inhibit not only the formation of the complexes but also to interfere with the electron flow from the complex to the cell membrane. The effect of IAA on active transport of potassium ions could consist in facilitating electron flow from ATP molecules to the carrier molecules in the membrane or to the sites in the membrane possessing higher affinity for potassium than for sodium ions.

A. Szent-Györgyi suggested that IAA can form charge transfer complexes exhibiting properties of electron conductor, which could agree with the role of IAA in active ion transport postulated here.

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SUMMARY

The effect of IAA on the uptake of Na²⁺ and K⁺ in cells of Nitella translucens was investigated. Ouabain at a concentration of 2.10⁻⁵ M was used as an inhibitor of active potassium transport. IAA at a concentration of 2.10⁻⁵ M induced a significant increase in potassium influx (0.732 pM.cm⁻².sec⁻¹ in the control, 1.224 in the medium containing auxin), while sodium influx remained unchanged. Tenfold higher concentration of IAA brought about a large decrease in potassium influx. Ouabain caused a drop in potassium influx in the variant with IAA and without it. A careful analysis of the data leads to the conclusion that IAA induced an increase in both active and passive potassium influx. Accepting Skočnik's mechanism of active potassium ions transport it is postulated that IAA stimulates the formation of the complex ATP·Mg₂·ATP-aze, which is responsible for active potassium transport. At the same time IAA can create charge transfer complexes.

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Stosunki jonowe i elektrofizjologia pojedynczych komórek Characeae

Cz. II Wpływ IAA na strumienie jonów sodu i potasu ze środowiska do komórek
Nitella translucens

Streszczenie

Badano wpływ IAA na pobieranie jonów Na$^{2+}$ i K$^{+}$ przez komórki Nitella translucens poddane działaniu IAA i ouabainy-selektywnego inhibitory aktywnego transportu jonów potasu. IAA w stężeniu 2.10$^{-5}$ M powoduje znaczný wzrost strumienia jonów potasu (0.732 pM.cm$^{-2}$.sec$^{-1}$ w kontroli, 1.212 w wariancie z IAA) nie zmieniając strumienia sodu. Dwudziesto-koło mnie większe stężenie IAA powoduje znaczný spadek strumienia. Ouabaina powoduje zmniejszenie strumienia jonów potasu zarówno w obecności IAA, jak i wobec braku auksyny w środowisku. Dokładna analiza uzyskanych danych prowadzi do wniosku, że IAA indukuje wzrost strumienia zarówno biernego, jak i czynnego jonów potasu w pojedynczych komórkach ramienic. Akceptując mechanizm aktywnego transportu jonów potasu zaproponowany przez Sko u wysunięto hipotezę, że IAA stymuluje powstawanie kompleksów ATP.MgQ, .ATP-aza odpowiedzialnych za aktywny transport jonów potasu, tworząc jednocześnie kompleksy z przenoszeniem ładunku.