Further study on the active transport of sodium across the motoneuronal membrane

M. Ito* and T. Oshima†

Department of Physiology, Australian National University, Canberra, Australia

Following a previous paper two methods were employed in a further investigation of the sodium pump in cat spinal motoneurons: (1) to compare the rate of sodium extrusion at different levels of the resting potential, and (2) to depress the sodium pump by the electrophoretic injection of metabolic inhibitors into the cells. The rate of sodium extrusion was found to be independent of the resting potential level, which suggests that the diffusion channels were not a main route for the sodium extrusion. A successful temporary block of the sodium pump was made by the intracellular injection of azide ions.

Introduction

It has been postulated by Coombs, Eccles & Fatt (1955a) that in cat spinal motoneurons the sodium pump operates to extrude sodium after the intracellular sodium concentration has been substantially raised by the electrophoretic injection of sodium, because the outward movement of sodium after the injection would be in opposition to the electrochemical gradient for this ion species across the cell membrane. Further theoretical support has been provided for this postulate by calculating that if the intracellular sodium concentration were to be restored to its pre-injection level by the diffusional efflux of sodium, the sodium conductance would have to be even larger than the total membrane conductance of motoneurons (Ito & Oshima 1964b). The rate of recovery after a sodium injection is so rapid that a very effective sodium pump must be postulated, hence depression of its action would be expected to result in large changes in the intracellular ionic concentrations.

The aim of this paper is to give experimental evidence for the contribution of the sodium pump both to the recovery from a sodium injection and to the dynamic maintenance of the intracellular ionic concentrations. First, since it can be assumed that diffusional ion movement, but not pump transport, is influenced by the electrical potential difference across the membrane, an investigation has been made of the influence that membrane potential has on the rate of recovery from a sodium injection. Secondly, an attempt is made to depress the sodium pump in motoneurons by the intracellular injection of metabolic inhibitors. This has been done most effectively by the injection of azide.

Methods

The experimental arrangements were the same as those described in previous papers (Ito & Oshima 1964a, b). Single-barrelled microelectrodes were mainly used.

* Present address: Department of Physiology, Faculty of Medicine, University of Tokyo, Japan.
† Present address: Section of Neurophysiology, Institute of Brain Research, School of Medicine, University of Tokyo, Japan.
and were filled with $2\,M$ sodium chloride, $3\,m$ sodium azide, $0.7\,m$ sodium fluoroacetate or $1\,m$ sodium iodoacetate solutions. The resistance of the microelectrodes ranged from 7 to 20 MΩ. Sodium salts of the metabolic inhibitors were used for filling the microelectrodes; hence sodium was injected by a depolarizing current and a metabolic inhibitor as an anion by a hyperpolarizing current from one and the same single microelectrode.

**RESULTS**

**Recovery time from sodium injections**

A previous paper (Ito & Oshima 1964b) dealt only with motoneurons with high resting potentials ($-72$ to $-86\,mV$) and large spike potentials ($82$ to $106\,mV$). In the present experiments a number of sodium injections were also made through NaCl-filled microelectrodes into cells with relatively low resting potentials ($-50$ to $-70\,mV$), which presumably were caused by the membrane damage inflicted by the impaling microelectrode. These injections were similarly employed for estimating the influence of the resting potential level upon the recovery process after a sodium injection.

Difficulties in analyzing the electrical activities of motoneurons at low resting potential levels arise from the fact that, following a sodium injection, the resting potential undergoes a relatively large change at a slow rate (Ito & Oshima 1964a). This change in the resting potential would modify such motoneuronal responses as the spike potential, the after-hyperpolarization following the spike and the inhibitory postsynaptic potential (IPSP), which, provided that the resting potential is constant, can be taken as indices of the intracellular ionic concentrations (Ito & Oshima 1964b). In figure 1 the following measurements have been plotted against the time before and after two trials of sodium injections: the resting potentials; the membrane potentials at the peaks of the spikes generated in the initial segment and the soma-dendritic regions of motoneurons ($IS$ and $SD$ spikes respectively in $A$); the maximum rates of rise and fall of the $SD$ spike ($E'_2$ and $E'_4$ respectively in $B$, cf. Ito & Oshima 1964b); the after-hyperpolarization following the $SD$ spike; and the IPSP ($C$). The distortion by the slow and large change in the resting potentials is indicated by the recovery time courses of these motoneuronal responses which deviate significantly from exponential curves that are regularly obtained at high membrane potentials (Ito & Oshima 1964b).

However, an approximate comparison of the respective recovery time courses from sodium injections can be made by determining the time intervals from the cessation of injection to the end-point of the recovery process, because at that time there was virtually a complete recovery of the membrane potential. These end-points were approximately given by the recovery curves and are indicated by the vertical arrows in figures 1$B$ and $C$. The recovery time, as it may be called, was measured after thirty-seven sodium injections by a current of $5 \times 10^{-8}\,A$ for $60\,s$, and is plotted in figure 2 as a function of the resting potential at the completion of recovery. It may be seen that there is no obvious correlation between the recovery time and the resting potential level ($P > 0.5$). This suggests that the mechanism
involved in the recovery process is not influenced by the electrical potential gradient across the cell membrane. The average recovery time amounted to $6.4 \pm 1.2$ min (s.d.).

**Figure 1.** Effects of sodium injections on motoneurons with low resting potentials. *A* and *B*, a posterior biceps-semitendinosus motoneuron. Single-barrelled NaCl-filled microelectrode. *A* to *E*, specimen records of antidromic spike potentials (upper traces) and their differentiated records (lower traces) taken before (*A*) and at 12(*B*), 90(*C*), 175(*D*) and 380s (*E*) after the injection. The ventral root stimulus was set just at threshold so that about half sweeps ran without antidromic invasion. Time scale, msec. Note that for the initial minute of recovery initial segment (IS)-soma-dendritic (SD) conduction was blocked (*A*). *B*: Plots of recovery of maximum rates of rise ($E'_g$) and fall ($E'_d$) of SD spike. Downward arrow in *B* indicates limit of recovery for $E'_g$. *C*, a plantaris motoneuron. Double-barrelled NaCl-filled microelectrode. The recovery curves for the IPSP evoked polysynaptically from a cutaneous (peroneal) volley (closed circles), for the after-potential (open circles) and for the resting potential (solid line) are illustrated, the termination of recovery on the former two curves being indicated by upward arrows. Note break in time scale. *f* to *h*, specimen records of the IPSP (stimulus indicated by a downward arrow) and the after-potential (stimulus indicated by an upward arrow) taken before (*f*), and at 60(*g*) and 495s (*h*) after the injection. Time scale, 10 ms. Horizontal arrows in *A*, *B* and *C* point the plottings at the pre-injection stages. Current for the sodium injections: $5 \times 10^{-8}$A for 60s.

*Injection of metabolic inhibitors*

Following antidromic ventral root stimulation in cats, Lloyd (1953) recorded from the ventral root very close to the spinal cord an after-potential which presumably originated from motoneuron somas (cf. Brooks, Downman & Eccles 1950). The post-asphyxial change in this after-potential (Fig. 2 of Lloyd 1953) closely resembled that in the intracellularly recorded motoneuronal after-potential following a sodium injection (see Fig. 1C, D of Ito & Oshima 1964b). With intracellular recording from cat motoneurons during asphyxiation (Kolmodin & Skoglund 1959) the spike potential and the after-potential also exhibited changes similar to those produced by a sodium injection. These observations suggest that
oxygen deficiency induces a rapid alteration in the intracellular cation concentrations, presumably due to block of the sodium pump. It may be expected that injection of a metabolic inhibitor into motoneurons would produce effects similar to those observed during asphyxia.

Azide

The after-effects of two successive injections of azide are illustrated in figures 3 and 4. The records in the first row in figure 3A were taken immediately after the microelectrode filled with 3mM NaN₃ solution was inserted into a motoneuron (Control-1). Further control records were taken about 20 min after the penetration (Control-2), revealing a reversal of the IPSP into a small depolarization, which presumably is due to azide diffusing out of the microelectrode tip (cf. Araki, Ito & Oscarsson 1961). After the first injection of azide, as shown in figure 4, there was an increase in the amplitude of the depolarizing IPSP, which was followed by an approximately exponential recovery with a time constant of about 20s. This effect is due to the free passage of the injected azide through the activated inhibitory subsynaptic membrane (Araki et al. 1961); hence the recovery curve for the IPSP expresses the diminution of the intracellular azide concentration. After the azide injection there was also a reduction of the spike potential and the after-hyperpolarization (figures 3A, 4) which recovered thereafter as the effect upon the IPSP diminished. Similar changes in the spike potential and the after-hyperpolarization might be seen to a very small extent in the control records taken long after the
penetration (Control-2) in comparison with the other control records (Control-1). The more prominent changes in the spike potential are observed after the second injection, when the azide dose was doubled (figure 3B). After the injection, as plotted in figure 4, the resting potential gradually decreased, while the spike potential recovered for 1 min and thereafter there was a slow deterioration. At 2 min, the $SD$ spike was abolished and the $IS$ spike still continued to decline. After about $3\frac{1}{2}$ min, however, the deterioration ceased and thereafter there was a slow but complete recovery which took a further 14 min. The following sodium injection (figures 3C, 4) had the typical effects which were previously reported (Ito & Oshima 1964b).

The depression of the spike potential immediately following the first azide injection occurred without appreciable change in the resting potential and diminished as the intracellular azide concentration was reduced, as indicated by the change of the IPSP; hence this effect possibly is due to a direct depressant action of azide on the mechanism generating the spike potential. On the other hand, following the second azide injection the spike and after-potential exhibited a slow deterioration (the second to fourth rows in figure 3B) which paralleled a progressive fall in the resting potential (figure 4). This combined effect seems to be

![Figure 3](image-url)

Figure 3. Comparison of effects between injections of azide (A, B) and sodium (C) ions. A plantar motoneuron was impaled with a NaN$_3$-filled single microelectrode. Antidromic spike potentials are shown on the left of A, B and C, while IPSPs polysynaptically evoked by a cutaneous (peroneal) volley (downward arrow in A) and after potentials (upward arrow in A) are illustrated on the right of each column. Approximate times after the respective injections are indicated in sec on the middle of each record. Voltage and time scales, 50 mV and 2 ms apply to the spikes; 5 mV and 10 ms to the IPSPs and the after-potentials.
Figure 4. Plotting of azide and sodium injections partly illustrated in figure 3. The resting potential, the membrane potential at the spike peak, the maximum rising ($E'_2$) and falling ($E'_4$) slopes of the spike, the after-potential and the IPSP are plotted as in figure 1.
due to a progressive increase of the intracellular sodium concentration and to the concomitant decrease of the intracellular potassium concentration, which would occur if the sodium pump was blocked effectively by the large dose of azide. The reversible and deleterious effects of azide were produced in seven injections into three motoneurons.

Throughout the period of impalement illustrated in figure 4, the IPSP remained as a depolarizing potential; hence a certain concentration of azide appears to have been attained in the cell by diffusion from the microelectrode tip. Nevertheless, the recovery following the sodium injection in figures 3C and 4 was much the same as in cells penetrated with NaCl-filled microelectrodes which were exclusively employed previously (Ito & Oshima 1964). Moreover, the first injection of azide apparently failed to inhibit the sodium pump. A certain threshold concentration of azide appeared to be needed for this blocking action. On the other hand, once the slow recovery was produced by a large dose of azide, the following recovery was greatly delayed; on the average after seven injections of azide, it took about 18 min for complete recovery. Since the azide concentration fell rapidly as indicated by the time course of the decrease in the depolarizing IPSP after each injection in figures 3A, B and 4, this delayed restoration might be due to a slow dissociation of azide from the respiratory enzymes. However, since the post-asphyxial recovery seems to have a slow time course (Lloyd 1953), it may alternatively be suggested that a slowing in metabolism occurs following its temporary block. It might be suspected that, since there was a later increase of the depolarizing IPSP at 4 to 16 min after the second azide injection (figure 4), the intracellular azide concentration was maintained high enough to keep the sodium pump partially depressed so as to delay the recovery. This, however, is unlikely, because, due to the rapid diffusion across the motoneuronal membrane, the concentration of azide would in this later stage have reached the low level determined by the diffusion out of the electrode tip (see above). The increase of the depolarizing IPSP would merely indicate a low concentration of potassium and a high concentration of chloride in the cell, the latter ions moving inward because of the reduced resting potential (cf. Coombs, Eccles & Fatt 1955).

One might expect a slower recovery from a sodium injection during the period of lowered activity of the sodium pump induced by an azide injection. Within 2 min immediately after an azide injection, sodium was injected into one and the same cell and the recovery of the sodium effects showed a longer recovery time (the mean of five trials; 8.2 ± 1.6 min) than that in the normal state. However, as described above, the azide action on a motoneuron is complicated in its time course by the slow recovery process following the temporary block of the sodium pump. Hence, when an azide injection is followed within 2 min by a sodium injection, the slow recovery might be merely due to delayed recovery from the azide injection as in the second injection of figures 3B and 4.

Other metabolic inhibitors

Fluoroacetate was injected intracellularly, but there were no marked effects upon the motoneuronal potentials. An example is illustrated in figure 5, where the
injection of fluoroacetate was repeated four times into a motoneuron without producing any appreciable effects. Iodoacetate may exert a depressant action on the spike potential similar to that obtained immediately after an azide injection, but the other potentials appeared to be hardly altered by an iodoacetate injection. Another metabolic inhibitor, 2-4-dinitrophenol, could not be applied intracellularly because of its extremely low solubility. It was also impossible to attain a sufficient intracellular concentration of cyanide because of its small acid dissociation constant (cf. Araki et al. 1961).

Another metabolic inhibitor, 2-4-dinitrophenol, could not be applied intracellularly because of its extremely low solubility. It was also impossible to attain a sufficient intracellular concentration of cyanide because of its small acid dissociation constant (cf. Araki et al. 1961).

Active sodium transport

**Figure 5.** Effects of fluoroacetate and sodium injections on a gastrocnemius-soleus motoneuron. The resting and spike potentials are illustrated as in figures 1 and 4. Specimen records, a to h, show the spikes (upper traces), and IPSPs and after-potentials (lower traces) at the times indicated by corresponding arrows on the resting potential record. Voltage scale 50 mV and time scale as 2 ms apply to the upper traces; 5 mV and time scale as 10 ms to the lower traces.

**Discussion**

The recovery time proposed in this paper can be used like the recovery time constant (Ito & Oshima 1964b) for characterizing the rate of removal of excess sodium from motoneurons after a sodium injection. In the comparable study of intracellular injections of various alkaline cations into motoneurons (Araki, Ito, Kostyuk, Oscarsson & Oshima 1962, 1964) an order of caesium > sodium > lithium was revealed in the rates of removal both by the recovery time constant and the recovery time.

We did not try to change the resting potential level by passing an extrinsic current across the cell membrane, but various resting potentials (—50 to —70 mV) were sampled from many trials of injection into many cells. Hence, it might be questioned if the observed insensitivity of the recovery time to the membrane
potential level is complicated by any injurious effect of the microelectrode. However, a control has been given by the recovery from a caesium injection which is found to vary according to the resting potential level (Araki et al. 1964). It is therefore possible to distinguish between the transport mechanisms for caesium and sodium; caesium transport across the motoneuronal membrane occurs, at least largely, via diffusion channels (Araki et al. 1964), while sodium is extruded chiefly by the sodium pump.

The high rate constant of the sodium extrusion from motoneurons may explain the high vulnerability of their electrical activities to oxygen lack (van Harreveld 1946; Brooks & Eccles 1947; Lloyd 1953; Gelfan & Tarlov 1955; Kolmodin & Skoglund 1959). This situation can be shown in the following approximate calculations.

The rate of change in the intracellular sodium concentration can be expressed by

\[
\frac{d[Na]_i}{dt} = \frac{m_i}{V_o} \frac{[Na]_i}{t_{Na}}
\]

(derived from Ito & Oshima’s (1964b) equations (17) and (21) on the assumption that the cell volume is constant). Here, \([Na]_i\) is the intracellular sodium concentration, \(m_i\) is the diffusional sodium influx, \(V_o\) is the cell volume, and \(t_{Na}\) is the recovery time constant from a sodium injection. The steady state under the normal condition is attained at \([Na]_i = [Na]_f\), \(d[Na]_i/dt = 0\). Then,

\[
\frac{m_i}{V_o} = \frac{[Na]_f}{t_{Na}}.
\]

It may be assumed that \([Na]_f = 15 \text{ mm} \) (Eccles 1957) and \(t_{Na} = 91 \text{ s} \) (Ito & Oshima, 1964b); hence \(m_i/V_o\) equals 15 mm/91 s. It follows that, if the pump is stopped suddenly, the second term on the right of equation (1) will diminish to zero (if \(t_{Na} = \infty\)) and the intracellular sodium concentration will start to increase at a rate of \(m_i/V_o\), i.e., at about 10 mm/min and there should be a corresponding decrease of intracellular potassium concentration. This estimate is in agreement with the actual time course of the onset of potential changes in cat motoneurons under asphyxia (Lloyd 1953; Kolmodin & Skoglund 1959) or subsequent to an intracellular azide injection. In the cerebral cortex, circulatory arrest induces rapid increase of electrical impedance of the brain material with swelling of apical dendrites and dominance of chloride in the cytoplasm of nerve cells (van Harreveld & Ochs 1956; van Harreveld & Schadé 1959). These changes were explained by the movement of sodium chloride from the extracellular to the intracellular space, but can also be understood as the results of depression of the sodium pump’s activity.

The authors wish to thank Professor Sir John Eccles, F.R.S. for his helpful comments on the manuscript.

**References**


