Biochimica et Biophysica Acta, 339 (1974) 234–245

BBA 76573

EFFECT OF ATP ON THE INTERMEDIARY STEPS OF THE REACTION OF THE ($Na^+ + K^+$)-DEPENDENT ENZYME SYSTEM

I. STUDIED BY THE USE OF *N*-ETHYLMALEIMIDE INHIBITION AS A TOOL

J. C. SKOU

Institute of Physiology, University of Aarhus, 8000 Aarhus C (Denmark)

(Received August 2nd, 1973)
(Revised manuscript received November 26th, 1973)

SUMMARY

1. It has been investigated how ATP, Na^+, K^+, and Mg^{2+} influence the inhibition of the ($Na^+ + K^+$)-activated enzyme system by *N*-ethylmaleimide.

2. ATP protects against the inhibition by *N*-ethylmaleimide. With ATP, without Mg^{2+} and EDTA, both Na^+ and K^+ increase the inhibitory effect of *N*-ethylmaleimide; K^+ has the effect in lower concentrations than Na^+. The main part of the Na^+ effect seems to be due to traces of Mg^{2+} in the enzyme preparation; these can be removed by EDTA, while EDTA does not influence the effect of K^+.

3. With ATP, Mg^{2+} increases the inhibitory effect by *N*-ethylmaleimide; Na^+ potentiates the effect by decreasing the requirement for Mg^{2+} for inhibition and it increases the inhibition which can be obtained by Mg^{2+}. K^+, on the other hand, increases the requirement for Mg^{2+}.

4. Without Mg^{2+}, with EDTA, K^+ decreases, while Na^+ gives a slight increase in the apparent affinity for ATP for the protection against *N*-ethylmaleimide.

5. With ATP, without Mg^{2+}, and with EDTA, the increase in inhibition due to K^+ can be eliminated by titration with Na^+ and vice versa. The curve for the elimination of the K^+ effect on the reaction with *N*-ethylmaleimide is identical with the curve for the activation of the catalytic activity by Na^+ in the presence of K^+ and no *N*-ethylmaleimide. The Na^+ concentration necessary for half maximum elimination of the K^+ effect on the inhibition by *N*-ethylmaleimide decreases with an increase in the ATP concentration.

6. ADP protects against *N*-ethylmaleimide like ATP; and an increase in the ADP concentration leads to a decrease in the concentration of Na^+ necessary for the elimination of the K^+ effect on the reaction with *N*-ethylmaleimide, but the concentration of ADP necessary to give a certain effect is about two times higher than that of ATP. ITP has practically no protective effect against *N*-ethylmaleimide. CTP and GTP have a low protective effect and a low effect on the apparent affinity for Na^+; 3 mM GTP or CTP has an effect of the same order of magnitude as 10 μM ATP.

7. The experiments suggest (1) that K^+ on the "Na^+-site", the i-site of the system (*i* for inside), decreases, while Na^+ at the same site gives a slight increase in the apparent affinity for ATP. (2) That the i-site exists in a K^+ and a Na^+ form, $i_P \rightleftharpoons i_S$, and due to the higher affinity for ATP of the Na^+ than of the K^+ form, ATP in the presence of Na^+ and K^+ shifts the equilibrium towards the Na^+ form. Without ATP, the "Na^+-site" has an apparent Na^+:K^+ affinity ratio of 0.4:1, while with saturating concentrations of ATP it is 3:1, i.e. ATP increases the apparent affinity for Na^+ relative to K^+ for the "Na^+-site" 7–8 times.

INTRODUCTION

N-Ethylmaleimide inhibits the ($Na^+ + K^+$)-activated enzyme system and the inhibitory effect is influenced by ATP, Na^+, and K^+ [1–4]. This opens a possibility that the reaction of the system with *N*-ethylmaleimide can be used as a tool to obtain information on the relationship between the effect of ATP, Na^+, and K^+ on the enzyme system. In the present paper such an attempt has been made. Some of the results have been given in a preliminary form [5–7].

METHODS

The enzyme was prepared from ox brain [8]; the specific activity of the g-strophanthin-sensitive activity was 160–220 μM P_i/mg protein per h (3 mM ATP, 3 mM Mg^{2+}, 120 mM Na^+, 30 mM K^+, pH 7.4, 37 °C). The g-strophanthin-insensitive activity which was 8–12% of the total activity with Mg^{2+} plus Na^+ plus K^+ was decreased to less than 0.5% of the g-strophanthin-sensitive activity by incubation of the enzyme preparation for 5 min to 2 h in 1 M NaSCN at 20 °C, pH 7.4 (Ottolenghi, P., personal communication). After the incubation, the enzyme preparation was washed 3 times with 0.25 M sucrose, 30 mM histidine–HCl, pH 7.4, by centrifugation. The sediment after the final centrifugation was resuspended in histidine–HCl and used as enzyme source. This treatment did not decrease the specific g-strophanthin-sensitive activity; on the contrary, in most preparations it gave a slight increase.

Reaction with N-ethylmaleimide

0.1–0.2 mg enzyme protein was incubated in 30 mM Tris–HCl, pH 7.4 at 37 °C with *N*-ethylmaleimide and combinations of ATP, Na^+, K^+, and Mg^{2+}. *N*-ethylmaleimide was added last, 10 s after the addition of enzyme. After the end of the incubation, 0.1 ml of the solution was transferred to 0.9 ml of the test solution which contained 30 mM Tris–HCl, pH 7.4, and with a final concentration of 3 mM Mg^{2+}, 3 mM ATP, 120 mM Na^+, 30 mM K^+, 1 mM β-mercaptoethanol with and without 10^{-4} M ouabain. The reaction was stopped by adding 0.1 mM trichloroacetic acid and P_i was measured according to Fiske and SubbaRow [9]. Control was enzyme which was incubated under the same conditions but without *N*-ethylmaleimide.

The figures show typical results, which have all been reproduced 3–5 or more times; for variability in the experimental results, see legends to Figs 9–11.

RESULTS

Preincubation of the enzyme system with *N*-ethylmaleimide at 37 °C leads to a decrease in activity (Fig. 1). The activity decreases fast for the first 10–20 min, and thereafter with a much slower rate, and it has not come to a steady state after 60 min of preincubation.

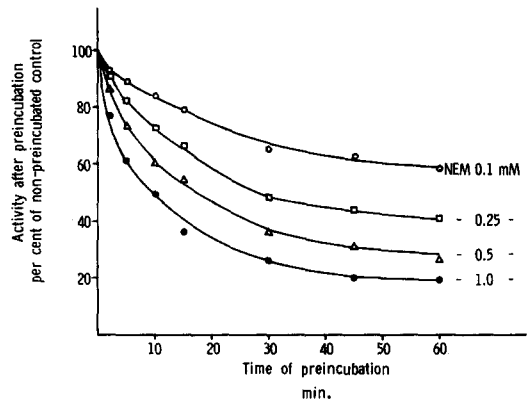


Fig. 1. The effect of different concentrations of *N*-ethylmaleimide (NEM) on the enzyme activity after different times of preincubation. The enzyme was in this and in the experiments shown in the following figures, apart from Fig. 11, preincubated in 30 mM Tris–HCl, pH 7.4, at 37 °C. After preincubation, the enzyme activity was tested in 30 mM Tris–HCl, pH 7.4, with 3 mM ATP, 3 mM Mg^{2+}, 120 mM Na^+ and 30 mM K^+.

ATP protects to a certain extent against the effect of *N*-ethylmaleimide (Fig. 2). After approx. 30 min of preincubation, the slope of the curve for the slow reaction with *N*-ethylmaleimide is nearly identical with and without ATP.

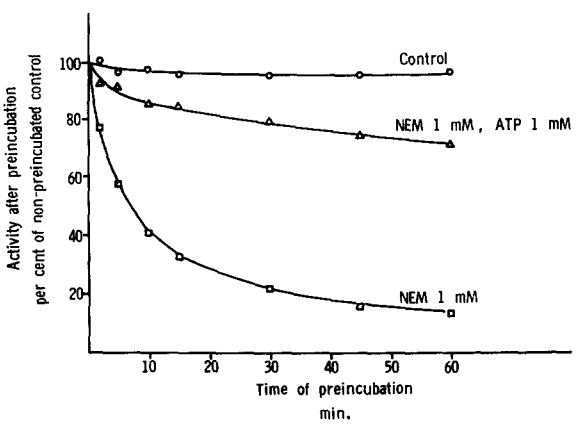


Fig. 2. The effect of 1 mM *N*-ethylmelaimide (NEM) with and without 1 mM ATP on the enzyme activity after different times of preincubation. Control is enzyme preincubated without *N*-ethylmaleimide and ATP.

In the following 30 min of preincubation and 1 mM of *N*-ethylmaleimide has been used.

With ATP in the preincubation medium, the addition of K^+ increases the reactivity of the system towards *N*-ethylmaleimide, and so does the addition of Na^+ (Fig. 3). K^+ has, however, an effect in lower concentrations than Na^+, and with saturating concentrations of the cations, the reactivity towards *N*-ethylmaleimide is higher with K^+ than with Na^+.

ADP protects to the same extent as ATP against the inhibition by *N*-ethylmaleimide. With ADP, Na^+ has, however, practically no effect on the inhibition, while the effect of K^+ is very nearly identical with the effect seen with ATP (Fig. 3).

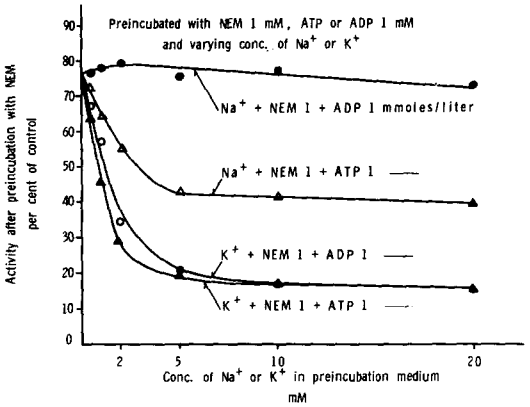
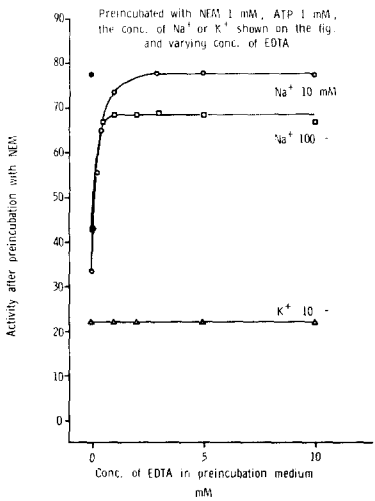


Fig. 3. The effect of Na^+ and of K^+ on the inhibition by *N*-ethylmaleimide (NEM) in the presence of ATP and of ADP. In this and the following figures apart from Fig. 11, the enzyme was tested after 30 min of preincubation.

The effect of Na^+ seen with ATP in the preincubation medium can to a certain extent be eliminated by EDTA. At the lower Na^+ concentrations, e.g. 10 mM, EDTA can completely overcome the Na^+ effect, while with higher Na^+ concentrations, e.g. 100 mM, a certain part of the Na^+ effect cannot be eliminated by EDTA (Fig 4). EDTA in the preincubation medium does not influence the effect of K^+ (Fig. 4). Nor has EDTA any effect on the protection by ATP without Na^+ or K^+ neither on the protection by ADP with or without Na^+ or K^+ (not shown).

In the presence of ATP, Na^+ increases the sensitivity of the system towards Mg^{2+} for the inhibition by *N*-ethylmaleimide (Fig. 5). Without Na^+, Mg^{2+} increases the inhibition by *N*-ethylmaleimide, but with Na^+, Mg^{2+} shows the effect in much lower concentrations and *N*-ethylmaleimide inhibits more strongly. Considering that there is 3 mM EDTA in the medium in the experiments in Fig. 5, the concentration of non-EDTA-bound Mg^{2+} necessary to give an effect in the presence of Na^+ is very low.

The experiment shown in Figs 4 and 5 suggest that Mg^{2+} is necessary for at least the main part of the Na^+ effect, and that the effect of Na^+ seen without added Mg^{2+} and EDTA is due to traces of Mg^{2+} in the enzyme preparation. The different levels of inhibition obtained with optimum concentrations of Mg^{2+} with and without



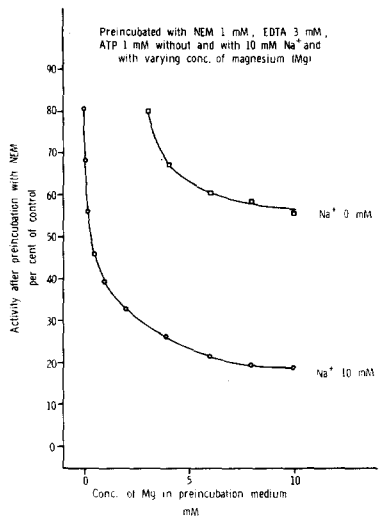


Fig. 4. The effect of EDTA on the inhibition by *N*-ethylmaleimide (NEM) in the presence of ATP and Na^+, and ATP and K^+. ●, the activity after preincubation with 1 mM *N*-ethylmaleimide, 1 mM ATP without Na^+, K^+, or EDTA.

Fig. 5. The effect of Mg^{2+} on the inhibition by *N*-ethylmaleimide (NEM) in the presence of ATP with and without Na^+.

Na^+ shows that the decrease in requirement for Mg^{2+} seen with Na^+ cannot solely be due to a higher affinity for Mg^{2+} of a Na^+-ATP form of the system.

With ATP without Na^+, K^+ increases the inhibitory effect of *N*-ethylmaleimide. Mg^{2+} is not required for the K^+ effect as it is for the Na^+ effect; on the contrary, Mg^{2+} seems to eliminate the K^+ effect. At a given K^+ concentration, the addition of Mg^{2+} decreases the inhibition by *N*-ethylmaleimide to the level found with Mg^{2+} without K^+ (Fig. 6), and the concentration of Mg^{2+} necessary increases with the K^+ concentration (not shown).

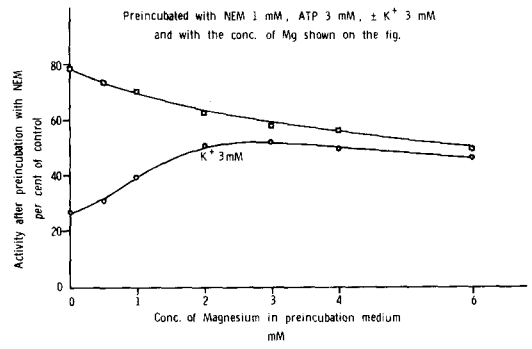


Fig. 6. The effect of Mg^{2+} on the inhibition by *N*-ethylmaleimide (NEM) with ATP, with and without K^+.

The effect of ATP depends on the K^+ concentration and vice versa. This is seen from Fig. 7, which shows the effect of ATP at different concentrations of K^+. Without K^+, the concentration of ATP necessary to give half maximum protection is about 20 μM under the conditions used. This value increases with the K^+ concentration, and at the same time the maximal protection which can be obtained by an increase in the ATP concentration decreases. It can also be seen from the figure that at a given ATP concentration the concentration of K^+ necessary to give a certain increase in inhibition is higher as the concentration of ATP increases.

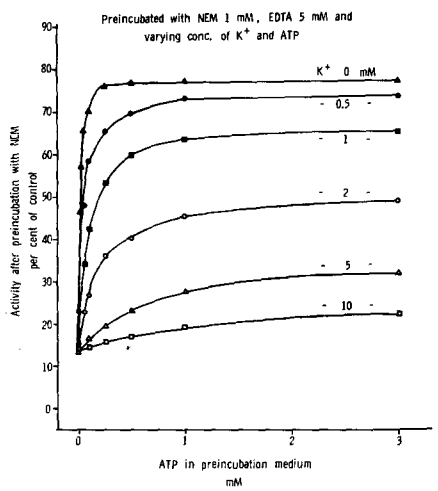


Fig. 7. The effect of ATP on the inhibition by *N*-ethylmaleimide (NEM) in the presence of different concentrations of K^+.

Na^+ has not the K^+ effect on the requirement for ATP; on the contrary, Na^+ gives a slight decrease in the requirement for ATP. With 5 mM EDTA and 100 mM Na^+, the concentration of ATP for half maximum protection was found to be about 12 μM, while it was about 20 μM without Na^+ and K^+ (not shown).

The effect of Na^+ on the inhibition seen in the presence of ATP both with and without EDTA can be eliminated by K^+. The concentration of K^+ necessary is low relative to the Na^+ concentration (Figs 8 and 9), and it increases with the Na^+ concentration (Fig. 8). In higher concentrations K^+ again increases the inhibition by *N*-ethylmaleimide and also for this effect the concentration necessary increases with the Na^+ concentration. K^+ can eliminate the effect of high concentrations of Na^+ which cannot be eliminated by EDTA (Fig. 9), cf. Fig. 4. It means that in the presence of high concentrations of Na^+, a small concentration of K^+ will decrease the inhibition by *N*-ethylmaleimide to the level found with ATP without Na^+ or K^+ in the preincubation medium.

It is, however, not only K^+ which can eliminate the Na^+ effect on the inhibition by *N*-ethylmaleimide. Na^+ can also eliminate the K^+ effect, and as can be seen in Fig. 8 the concentration of Na^+ necessary increases with the K^+ concentration.

In Fig. 9 is shown how a variation in the Na^+ and K^+ concentrations in the preincubation medium influences the *N*-ethylmaleimide inhibition in the presence of

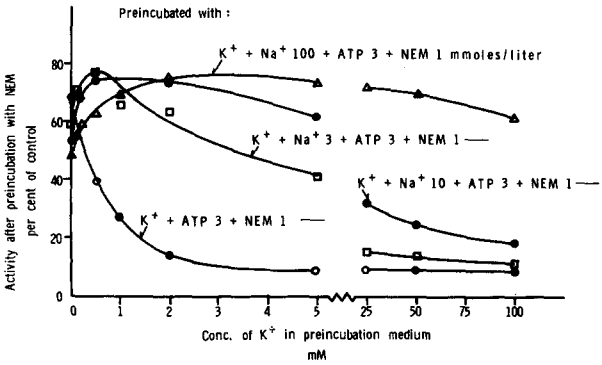


Fig. 8. The effect of K^+ on the inhibition by *N*-ethylmaleimide (NEM) in the presence of ATP and different concentrations of Na^+.

3 mM ATP, 1 mM *N*-ethylmaleimide and 5 mM EDTA; the concentration of Na^+ and K^+ has been kept constant at 150 mM. For comparison is shown in Fig. 10 how the same variation in the Na^+ and K^+ concentrations influences the hydrolytic activity of the system in the presence of 3 mM ATP and 3 mM Mg^{2+} without *N*-ethylmaleimide. It must be emphasized that in Fig. 9 it is the concentration of Na^+ and K^+ in the preincubation medium which has been varied, and the activity has in all cases been tested with Na^+ 120 mM, K^+ 30 mM, ATP 3 mM, and Mg^{2+} 3 mM in the medium; in Fig. 10 the enzyme has not been preincubated, it is the concentrations of Na^+ and K^+ in the test medium which have been varied, and besides Na^+ and K^+ the medium contains 3 mM ATP and 3 mM Mg^{2+}.

A comparison between Fig. 9 and Fig. 10 shows that the two curves follow the same pattern. The Na^+:K^+ concentration ratio for half maximum inhibition by *N*-ethylmaleimide, 36:114, is identical with the concentration ratio for half maximum activation of the catalytic activity, 37:113. For the right part of the curves, the concentration of K^+ necessary to eliminate the Na^+ effect on the inhibition by *N*-ethylmaleimide is of the same order as the K^+ concentration necessary to activate the catalytic activity in the presence of Na^+.

ATP decreases the concentration of Na^+ necessary for half maximum elimination of the K^+ effect on the inhibition (Fig. 11). Without ATP the concentration of Na^+ is approx. 108 mM in the presence of 42 mM K^+. It decreases with an increase in the ATP concentration, and with the concentration of ATP which gives maximum effect, 3 mM, the Na^+:K^+ concentration ratio at half maximum elimination of the K^+ effect is 36:114, (Fig. 9). ATP also decreases the inhibitory effect of *N*-ethylmaleimide in the presence of Na^+ and of Na^+ plus K^+ while with K^+, higher concentrations of ATP give a slight increase in the inhibition (Fig. 11).

In Fig. 12 is shown the effect of ATP on the inhibition by *N*-ethylmaleimide at different Na^+:K^+ concentration ratios. At a high ratio, 125:25 mM, the curve is hyperbolic, while at lower ratios, 50:100 and 25:125 mM, it becomes s-shaped. The concentration of ATP for half maximum effect decreases with an increase in the Na^+:K^+ ratio and at 125:25 mM the ATP concentration necessary is about the same as with Na^+ without K^+.

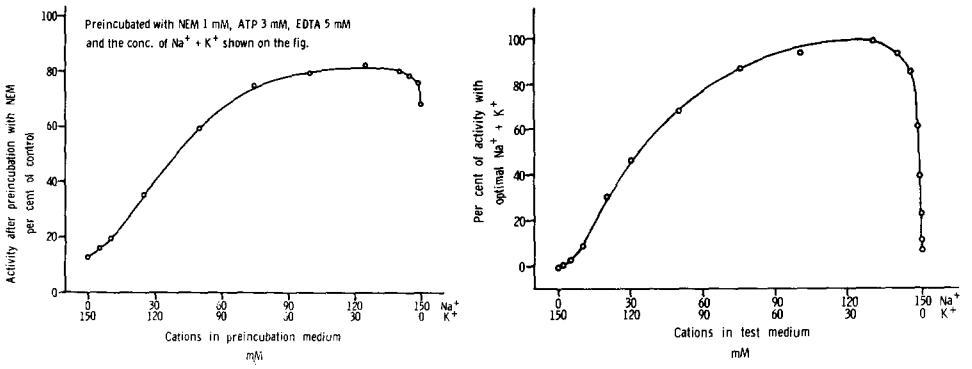


Fig. 9. The effect of a variation in the Na^+ and K^+ concentration on the inhibition by *N*-ethylmaleimide (NEM) in the presence of 3 mM ATP. The sum of the concentration of Na^+ and K^+ has been kept constant at 150 mM. In three such experiments the mean concentration of $Na^+ \pm$S.E. for half maximum elimination of the K^+ effect (the left part of the curve) was 36 $\pm$ 0.4 mM ($n = 3$) ($Na^+ + K^+ = 150$ mM).

Fig. 10. The effect of a variation in the concentration of $Na^+ + K^+$ on the catalytic activity of the enzyme system. In these experiments the enzyme has not been preincubated, but the activity has been tested with 3 mM ATP, 3 mM Mg^{2+}, and varying concentrations of Na^+ and K^+ in the test medium, and without *N*-ethylmaleimide (NEM). In three such experiments the mean concentration of $Na^+ \pm$S.E. for half maximum activation (the left part of the curve) was 37 $\pm$ 0.6 mM ($n = 3$) ($Na^+ + K^+ = 150$ mM).

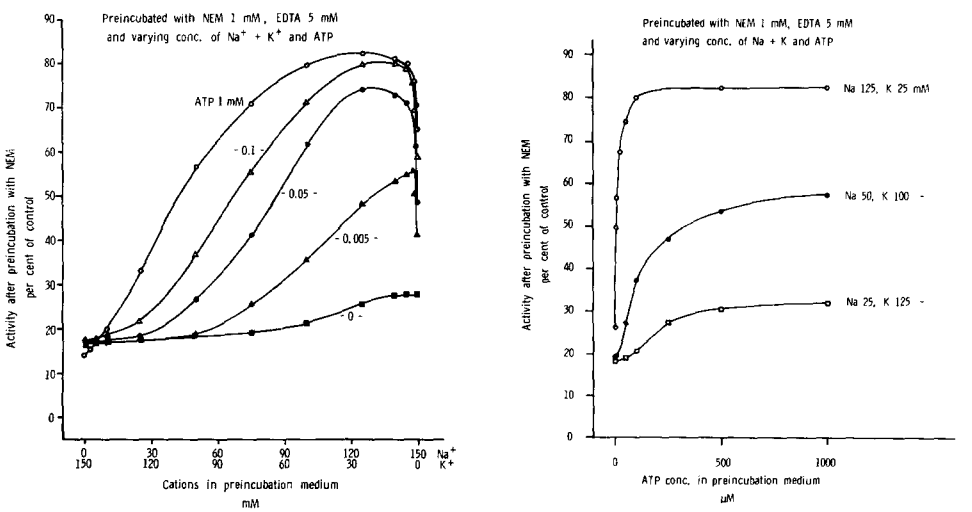


Fig. 11. The effect of a variation in the concentrations of $Na^+ + K^+$ on the inhibition by *N*-ethylmaleimide (NEM) with different concentrations of ATP. From a number of such experiments the mean concentration of $Na^+ \pm$ S.E. for half maximum elimination of the K^+ effect was for 1 mM ATP 42 $\pm$ 0.6 mM ($n = 3$); for 0.1 mM ATP 64 $\pm$ 0.4 mM ($n = 3$); for 0.05 mM ATP 79 $\pm$ 1.6 mM ($n = 6$); for 0.005 mM ATP 101 $\pm$ 0.6 mM ($n = 3$); and without ATP 108 $\pm$ 2.5 mM ($n = 5$) ($Na^+ + K^+ = 150$ mM).

Fig. 12. The effect of a variation in the concentration of ATP on the inhibition by *N*-ethylmaleimide (NEM) in the presence of different concentrations of $Na^+ + K^+$.

As it was shown in Fig. 3, ADP protects against *N*-ethylmaleimide, and this effect was influenced by K^+ in the same manner as with ATP, while Na^+ had no effect. With ADP, Na^+ can eliminate the effect of K^+ as it can with ATP, and the concentration of Na^+ necessary decreases with an increase in the concentration of ADP as it does with ATP (Fig. 13). But the concentration of ADP necessary to give a certain effect is about 2 times higher than the concentration of ATP (Fig. 14).

ITP, CTP, and GTP have a very low protective effect against *N*-ethylmaleimide. With 3 mM ITP and 3 mM CTP, the effect is lower than with 10 μM ATP, and with 3 mM GTP it is slightly higher (Fig. 15); their effect on the Na^+ concentration neces-

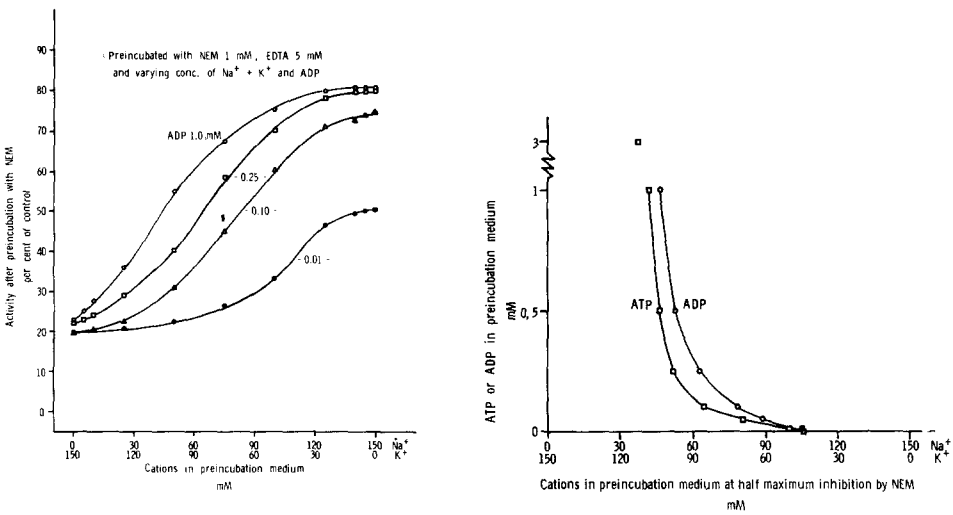


Fig. 13. The effect of a variation in the concentrations of $Na^+ + K^+$ on the inhibition by *N*-ethylmaleimide (NEM) in the presence of different concentrations of ADP.

Fig. 14. The effect of ATP and ADP on the $Na^+ : K^+$ concentration ratio for half maximum elimination of the K^+ effect on the inhibition by *N*-ethylmaleimide (NEM) ($Na^+ + K^+ = 150$ mM).

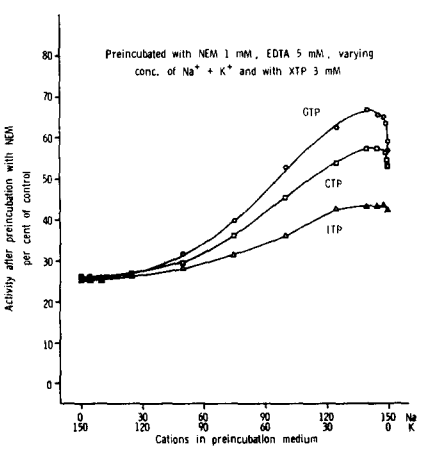


Fig. 15. The effect of ITP, CTP, and GTP on the inhibition by *N*-ethylmaleimide (NEM) at varying concentrations of $Na^+ + K^+$.

sary to give half maximum protection against the K^+ effect on the inhibition corresponds with their lower protective effect, cf. Fig. 15 and Fig. 11.

DISCUSSION

The reaction with *N*-ethylmaleimide does not go to an equilibrium, and the reaction is not reversible. It seems to consist of a relatively fast phase followed by a slower, and the effect of the different ligands on the inhibition seems mainly to be on the fast phase.

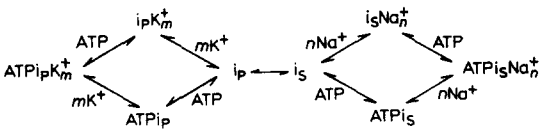
There is a parallelism between the effect of ATP, Na^+, and K^+ on the inhibition by *N*-ethylmaleimide and on the catalytic activity of the system; furthermore, the effect of Na^+ and K^+ on the affinity for ATP for protection against inhibition by *N*-ethylmaleimide goes parallel to the effect on the binding of ATP to the enzyme system [10, 11], and the effect of ATP, Na^+, and K^+ on the requirement for Mg^{2+} for the inhibition by *N*-ethylmaleimide goes parallel to the effect on the requirement for Mg^{2+} for the reaction with g-strophanthin [12]. Finally ATP has a parallel effect on the apparent affinity for Na^+ relative to K^+ both for the inhibition by *N*-ethylmaleimide (see below) and for the catalytic activity (see the following paper [13]). It suggests that *N*-ethylmaleimide in the concentration used does not influence the equilibrium between the different forms set by the ligands; but the different forms are inhibited differently by *N*-ethylmaleimide. This makes it possible to use the inhibition by *N*-ethylmaleimide as a tool to obtain information about the effect of the ligands also under conditions where there is no inhibition by *N*-ethylmaleimide and especially to obtain information about the interplay between K^+, Na^+ and ATP without Mg^{2+} in the medium.

ATP decreases the Na^+:K^+ ratio for half maximum elimination of the K^+ effect on the inhibition by *N*-ethylmaleimide, (the left part of the curves in Fig. 11). Without ATP the Na^+:K^+ ratio for half maximum effect is 108:42 while with saturating concentrations of ATP it is 36:114 (Fig. 9). It suggests that ATP increases the apparent affinity of the system for Na^+ relative to K^+ from about 0.4:1 (108:42) without ATP to 3:1 (37:113) with saturating concentrations of ATP, i.e. 7–8 times.

With 3 mM ATP the curve which show the decrease in *N*-ethylmaleimide inhibition with an increase in the Na^+:K^+ concentration ratio is similar to the curve which show the increase in catalytic activity (the left part of the curves in Figs 9 and 10); the Na^+:K^+ concentration for half maximum effect on the *N*-ethylmaleimide inhibition and on the catalytic activity was the same (36:114 and 37:113). The increase in catalytic activity seen when the Na^+:K^+ concentration ratio is increased is due to a replacement of K^+ by Na^+ on the site where Na^+ activates, the "Na^+-site" of the system. This site is facing the internal solution in the intact cell [14–16], the i-site. The similarity between the two curves suggests that the Na^+ elimination of the K^+ effect on the *N*-ethylmaleimide inhibition is also due to an effect of Na^+ on the "Na^+-site". This means that it is the apparent affinity for Na^+ relative to K^+ on the "Na^+-site" of the system which is increased 7–8 times by ATP.

K^+ decreases while Na^+ gives a slight increase in the apparent affinity for ATP for the protection against *N*-ethylmaleimide and ATP decreases the apparent affinity for K^+. With Na^+ plus K^+ an increase in the Na^+:K^+ concentration ratio leads to an increase in the apparent affinity for ATP parallel with a decrease in the *N*-ethylmalei-

mide inhibition, compare Figs 9–12. According to the above discussed it suggests that it is also the replacement of K^+ by Na^+ on the "Na^+-site" which leads to the increase in the apparent affinity for ATP; this means that it is K^+ on the "Na^+-site" which gives the decrease in the apparent affinity for ATP and vice versa. An apparent affinity for ATP with the i-site on the K^+ form which is lower than with the i-site on the Na^+ form can explain that ATP increases the apparent affinity for Na^+ relative to K^+ for this site of the system. It suggests the following scheme for the reaction where the i_P form has a lower affinity for ATP than the i_S form (P for potassium and S for sodium). According to the scheme ATP influences the equilibrium between a Na^+ and a K^+ form of the system, and not as suggested by Post et al. [17] the rate by which K^+ is displaced by Na^+.



The effect of ATP on the Na^+:K^+ ratio for protection by Na^+ is measured under conditions where the concentration of K^+ is high enough to saturate the site where potassium activates, the "K^+-site". This site is facing the external solution in the intact cell [14–16], the o-site .Assuming that the o-site and the i-site exist simultaneously [18], this means that the effect of ATP is on the equilibrium between a $^0K_m^+/^iK_n^+$ and a $^0K_m^+/^iNa_n^+$ form of the system. Without ATP, the equilibrium between the two forms seems to be towards $^0K_m^+/^iK_n^+$, and it is due to the reaction with ATP (without magnesium) that it is shifted towards $^0K_m^+/^iNa_n^+$.

The different inhibition by *N*-ethylmaleimide of the $^0K_m^+/^iK_n^+$ and the $^0K_m^+/^iNa_n^+$ form of the system in the presence of ATP suggests a different conformation of the two forms; this may mean that the o-site in the K^+ form interacts with the i-site in the K^+ form o_P/i_P, in a way which differs from that of the interaction with the i-site in the Na^+ form, o_P/i_S.

Of the triphosphates, the effect on the apparent Na^+:K^+ affinity ratio is relatively specific for ATP. ITP has practically no effect, and CTP and GTP has a certain effect, but in concentrations which are about 100 times higher than ATP. ADP, on the other hand, has an effect like ATP in a concentration which is about 2 times higher. AMP has no effect (not shown). This difference in the effect of ATP, ITP, GTP, CTP, and ADP on the affinity ratio is of the same order of magnitude as the differences found in their abilities to bind to the system [19].

ATP and ADP has thus about the same effect on the affinity for Na^+ versus K^+ on the i-site of the system. They differ, however, in that ATP in the presence of Na^+ decreases the requirement for Mg^{2+}, which ADP does not, and that ATP can be hydrolyzed.

ACKNOWLEDGEMENT

The author is indebted to Mrs Hanne Ouertani and Miss Bitten Holm for excellent technical assistance.

REFERENCES

1 Skou, J.C. (1963) Biochem. Biophys. Res. Commun. 10, 79–84
2 Skou, J. C. and Hilberg, C. (1965) Biochim. Biophys. Acta 110, 359–369
3 Banerjee, S. P., Wong, S. M. E., Khama, V. K. and Sen, A. K. (1972) Mol. Pharmacol. 8, 8–17
4 Banerjee, S. P., Wong, S. M. E. and Sen, A. K. (1972) Mol. Pharmacol. 8, 18–29
5 Skou, J. C. (1965) Proc. XXIII Int. Congr. Physiol. Sci., Tokyo, 578–586
6 Skou, J. C. (1969) in The Molecular Basis of Membrane Function (Tosteson, D. C., ed.), pp. 455–482, Prentice-Hall, New Jersey
7 Skou, J. C. (1972) in Mitochondria/Biomembranes (van Deenen et al., eds), pp. 339–352, North Holland Publ. Comp., Amsterdam
8 Skou, J. C. and Hilberg, C. (1969) Biochim. Biophys. Acta 185, 198–219
9 Fiske, C. H. and SubbaRow, Y. (1925) J. Biol. Chem. 66, 375–400
10 Nørby, J. G. and Jensen, J. (1971) Biochim. Biophys. Acta 233, 104–116
11 Hegyvary, C. and Post, R. L. (1971) J. Biol. Chem. 246, 523–5240
12 Skou, J. C., Butler, K. W. and Hansen, O. (1971) Biochim. Biophys. Acta 241, 443–461
13 Skou, J. C. (1974) Biochim. Biophys. Acta 339, 246–257
14 Glynn, I. M. (1962) J. Physiol. London 160, 18P
15 Laris, P. C. and Letchworth, P. E. (1962) J. Cell. Comp. Physiol. 60, 229–234
16 Whittam, R. (1962) Biochem. J. 84, 110–118
17 Post, R. L., Hegyvary, C. and Kume, C. (1972) J. Biol. Chem. 247, 6530–6540
18 Skou, J. C. (1973) J. Bioenerg. 4, 203–232
19 Jensen, J. and Nørby, J. G. (1971) Biochim. Biophys. Acta 233, 395–403