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# Myometrial ($Na^+ + K^+$)-activated ATPase and its $Ca^{2+}$ sensitivity

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**Ouabain-sensitive ($Na^+ + K^+$)-ATPase activity in the rat myometrial microsome fraction could only be determined following detergent treatment. The ($Na^+ + K^+$)-ATPase activity manifested by detergent treatment proved very stable even to high concentrations of $NaN_3$, in contrast $Mg^+$-ATPase activity was reduced to about 30 percent of the control. The major part of the $Mg^{2+}$-ATPase in the myometrial membrane preparation was found to be identical with the $NaN_3$-sensitive ATP diphosphohydrolase capable of ATP and ADP hydrolysis. This monovalent-cation-insensitive ATP hydrolysis could be extensively reduced by DMSO. Furthermore DMSO prevented the inactivation of the ($Na^+ + K^+$)-ATPase activity. 10–100 μM $Ca^{2+}$ inhibited the ($Na^+ + K^+$)-ATPase activity obtained in the presence of SDS by 15–50 percent. The $Ca^{2+}$ sensitivity of the enzyme was considerably decreased if the proteins solubilized by the detergent had been separated from the membrane fragments by ultracentrifugation. The inhibitory effect could be regained by combining the supernatant with the pellet. $Ca^{2+}$ sensitivity of the ($Na^+ + K^+$)-ATPase activity was preserved even after removal of the solubilized proteins provided that DMSO had been applied. It appears that a factor in the plasma membrane solubilized by SDS may be responsible for the loss of $Ca^{2+}$ sensitivity of the ($Na^+ + K^+$)-ATPase activity, the solubilization of which can be prevented by DMSO.**

## Introduction

In most tissues only a portion of the total ($Na^+ + K^+$)-ATPase capacity may be determined unless the isolated membranes have been previously treated with different detergents. It is extremely difficult to characterise this enzyme in plasma membrane fragments isolated from various smooth muscles [1–6] because the ouabain sensitive ($Na^+ + K^+$)-ATPase activity is very low compared to the $Mg^{2+}$-ATPase activity. In some tissues rabbit aorta, rabbit and rat myometrium no activity could be determined at all [2,3,5], although the functioning and physiological relevance of the ($Na^+ + K^+$)-pumps was shown in smooth muscle, as well [9]. Recently it has been reported that the high activity ATP hydrolysis in the presence of $Mg^{2+}$ is due to the ATP diphosphohydrolase (EC 3.6.1.5), the enzyme which is capable of cleaving ATP as well as ADP. This enzymatic activity demonstrated in a number of tissues is azide sensitive in each case [10–12].

It is well known that different detergent treatments can lead enhancement of the ($Na^+ + K^+$)-ATPase activity in certain tissues [13–16]. Most of the detergents inhibit the enzyme at higher concentrations [17]. A large excess of bovine serum albumin added to the membrane proteins has been shown to protect the enzyme against the inactiva-

Abbreviations: DMSO, dimethyl sulfoxide; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*'-tetraacetic acid; SDS, sodium dodecyl sulfate; $(Ap)_5A$, penta[adenylyl-(3 → 5)] adenosine; ATPase, adenosine triphosphatase.

tion caused by higher concentrations of detergents, in this way the determination of the $(Na^+ + K^+)$-ATPase activity becomes more reliable [18,19]. The effect of detergents is in general interpreted as a manifestation of the latent $(Na^+ + K^+)$-ATPase activity, for which several mechanism have been proposed [20,21].

A possible explanation of the stimulatory effect of the detergents is the solubilization of some inhibitor or repressor molecules. A membrane-bound inhibitor has been isolated from electric organ [22] and detected in various other tissues as well [23,24]. Thus a possible role of certain endogenous inhibitors regulating the enzyme function can be supposed.

$Ca^{2+}$ inhibits the $(Na^+ + K^+)$-ATPase activity in various tissues at relatively high, millimolar concentrations [25,26]. On the other hand, Powis et al. [27] found that less than 1 $\mu$M $Ca^{2+}$ increases the $(Na^+ + K^+)$-ATPase activity of rat brain homogenate. Furthermore data obtained in smooth muscle plasma membrane preparations suggest that the membrane bound $Ca^{2+}$ may also play a role in influencing the $(Na^+ + K^+)$-ATPase activity [28].

We intended to characterize the rat myometrial plasma membrane bound $(Na^+ + K^+)$-ATPase and required the development of a feasible procedure for the manifestation and determination of the enzyme activity. Furthermore, we examined the possibility that a factor solubilized by detergent from the plasma membrane of myometrium may play a role in the extremely high $Ca^{2+}$ sensitivity of the enzyme.

## Materials and Methods

*Isolation of the microsome fraction.* Myometrium of 150–200 g CFA rats pretreated with 100 $\mu$g diethylstilboestrol for 3 days was isolated by trimming the fat and connective tissue and scraping the endometrium off the uteri. The myometrium strips were minced and homogenized in 10 volumes of homogenization buffer (0.25 M sucrose and 50 mM histidine-HCl or Tris-HCl pH 7.2) in Ultra-Turrax homogenizer (Janke-Kunkel KG IKA-WERK, F.R.G.) at 3/4 of the maximum speed at 0–4°C for 90 s.

The homogenate was filtered through a double layer cheese cloth and centrifuged at $10000 \times g$ for 20 min. The supernatant was subjected to centrifugation at $100000 \times g$ for 60 min. The microsomes sedimented in the pellet were suspended in 50 mM histidine-HCl or Tris-HCl (pH 7.2) buffer and stored at −20°C.

*Preparation of deoxycholate (DOC)-treated microsomes.* The deoxycholate-treated microsomes fraction was prepared according to the method of Klodos et al. [29], with several modifications. The procedure in detail was as follows: The 10% homogenate was centrifuged at $10000 \times g$ for 20 min and the pellet rehomogenized in a Teflon-glass homogenizer. Sodium deoxycholate was added so that the deoxycholate/protein ratio fell between 0.08 and 0.1 (0.15–0.25% (w/v)) and the suspension stirred gently at 0°C for 30 min, and then centrifuged at $10000 \times g$ for 20 min. The microsomes were sedimented from the supernatant by centrifugation of $100000 \times g$ for 60 min, suspended in 50 mM histidine-HCl buffer (pH 7.2), and stored at −20°C.

*SDS-treatment of microsomes.* Myometrial microsomes prepared as described above were subjected to SDS treatment as published by Jørgensen [30], that is: 1 ml aliquots of the microsomes containing 1 mg protein were incubated with freshly dissolved recrystallized SDS at 20–23°C for 10 min. The SDS/protein ratios applied are indicated in the legends of figures. In order to separate the solubilized components (S-Sn) from the membranous fraction (S-P), the SDS-treated microsomes were centrifuged at $100000 \times g$ for 60 min. The pellet was suspended in 50 mM histidine-HCl (pH 7.2).

*DMSO-treatment of microsomes.* 5–50% (v/v) DMSO was slowly added to the microsomes at 1 mg/ml protein concentration with gentle stirring, low temperature was maintained 0–4°C during the addition. Following a 10 min incubation at 20–23°C, aliquots were subjected to ATPase activity measurements or the above described SDS treatment.

*Determination of enzyme activities.* $Mg^{2+}$-ATPase activity was measured in the presence of 5 mM $MgCl_2$, 5 mM ATP ($Na^+$-free, neutralized by imidazole) and 50 mM Tris-HCl (pH 7.4). The assay mixture for the determination of $(Na^+ + K^+)$-ATPase activity contained 100 mM NaCl, 20 mM KCl and if tested 1 mM ouabain in addition

to the above components. Free $Ca^{2+}$ concentrations up to 150 $\mu$M were set using EGTA buffer system and calculated by the method of Bartfai [31].

ATP-diphosphohydrolase activity was determined in the assay mixture as for $Mg^{2+}$-ATPase activity measurement except that ADP was substituted for ATP. Myokinase (adenylate kinase) activity of myometrial microsomes heated at 90°C for 5 min [32] was measured by comparing $P_i$ release from ATP and ADP in the presence for an externally added highly active pure $(Na^+ + K^+)$-ATPase isolated from the outer medulla of pig kidney by the method of Jørgensen [30]. In addition, the effect of $(AP)_5A$, a specific myokinase inhibitor on the $P_i$ release in the presence of ATP and ADP was tested at 1 mM concentration of the inhibitor. The volume of the reaction mixture was 1 ml in every case. After incubation at 37°C for 30 min, 100 $\mu$l of 60% trichloroacetic acid was added and the $P_i$ liberated was measured [33]. Protein content was determined according to the method of Lowry et al. [34]. Bovine serum albumin was used as a standard protein.

*Chemicals.* Sodium deoxycholate was purchased from Merck. DMSO was from Fluka. EGTA was obtained from Serva. Bovine serum albumin was Sigma's product. $(Ap)_5A$ was purchased from Boehringer-Mannheim F.R.G. SDS recrystallized was from Reanal. The chemicals not listed were reagent grade from Reanal.

## Results

*The effects of detergent treatments on the myometrial microsome fraction*

ATPase activities of the control and detergent treated microsomes are summarized in Table I. The control microsome fraction exhibited no ouabain sensitive $(Na^+ + K^+)$-ATPase activity. It possessed an extremely high $Mg^{2+}$-ATPase activity (100 $\mu$mol $P_i$/h per mg). A slight ouabain-insensitive monovalent cation activation occurred in microsomes isolated in histidine-HCl containing medium, however, in microsomes prepared in Tris-HCl containing medium monovalent cations did not activate ATP hydrolysis, on the contrary this inhibited it by approximately 20%. Dependence of ATPase activity on the isolation medium has been reported by other authors as well [4]. The inhibitory effect of monovalent cations was also observed in DOC-treated microsomes isolated in histidine-

TABLE I

ATPase ACTIVITIES OF MYOMETRIAL MICROSOMES PREPARED UNDER DIFFERENT CONDITIONS

Microsome fractions were isolated and stored in homogenization buffer adjusted with 50 mM Tris-HCl or 50 mM histidine-HCl (pH 7.2). DOC treatment was carried out as described in Materials and Methods. At the treatment with SDS 1 mg microsomal protein was preincubated with 0.35 mg SDS, then centrifuged at 100000 × *g* for 60 min and ATPase activity of 25 $\mu$g of the pellet (S-P) was measured.

| Condition of treatment | ATPase activity (mean ± S.D.) ($\mu$mol $P_i$/h per mg) | | | |
|---|---|---|---|---|
| | $+Mg^{2+}$ | $+Mg^{2+}+Na^+ + K^+$ | $+Mg^{2+}+Na^+ + K^+$ + ouabain | ouabain-sensitive activity |
| Control in Tris-HCl | 118 ± 14 | 93 ± 13 | 93 ± 13 | 0 ($n$ = 12) |
| Control in histidine-HCl | 90 ± 11 | 97 ± 13 | 97 ± 12 | 0 ($n$ = 12) |
| DOC-treated in histidine-HCl | 104 ± 13 | 79 ± 13 | 69 ± 7 | 10,6 ± 0,9 ($n$ = 9) |
| SDS-treated in histidine-HCl | 33 ± 13 | 43 ± 10 | 33 ± 11 | 10,5 ± 0,7 ($n$ = 8) |

HCl containing medium. However it is worth mentioning that the ATPase activity of the DOC-treated microsome fraction proved partially ouabain sensitive in the presence of $Na^+$ and $K^+$. $Mg^{2+}$-ATPase activities either of the control or the treated samples were ouabain insensitive, data not shown.

SDS treatment proved suitable for the determination of the ouabain sensitive ($Na^+$+ $K^+$)-activated ATPase activity. $Mg^{2+}$-ATPase activity in the SDS-treated microsomes was reduced to 35–40% of the control, the inhibitory effect of monovalent cations was eliminated, moreover a $Na^+$- and $K^+$-dependent ATP hydrolysis which was completely inhibited by ouabain could be measured.

*The effect of $NaN_3$ and DMSO on the ATPase activities*

The high $Mg^{2+}$-ATPase activity of the control and the DOC-treated microsome fraction was due for the most part to the ATP diphosphohydrolase (EC 3.6.1.5), an enzyme already reported in several tissues [10–12]. As shown in Fig. 1, $P_i$ release from ATP and ADP could be measured and descended steeply as a function of the $NaN_3$ concentration.

Virtual ADP hydrolysis might also be due to myokinase activity, provided that the ATP synthetized by myokinase is hydrolyzed by ATPases present. There was, however, no myokinase activity in our microsome fraction when tested by either a specific inhibitor $(AP)_5A$ or a coupled enzyme assay (see Materials and Methods, data not shown). Monovalent cations (100 mM $Na^+$+ 20 mM $K^+$) inhibited the ATP hydrolysis in microsomes prepared in the presence of Tris buffer as well as in the DOC-treated preparation (Table I). This inhibitory effect was not only eliminated by addition of $NaN_3$ but a ouabain sensitive ($Na^+$

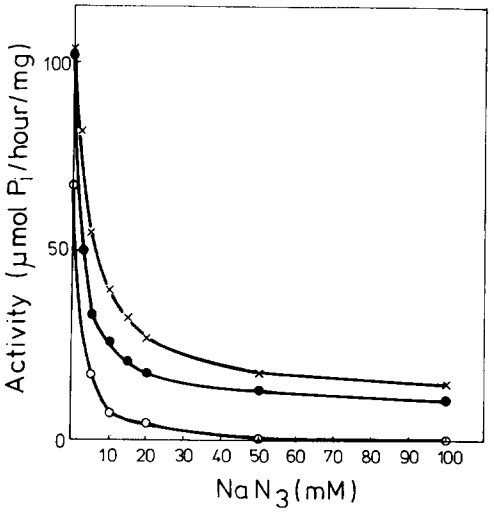


Fig. 1. Azide inhibition curves of the $Mg^{2+}$-dependent ATP and ADP hydrolysis of myometrial microsome fraction. 25 µg of microsomes or DOC-treated microsomes were preincubated with various concentrations of $NaN_3$ without ATP or ADP in assay mixtures at 0°C for 10 min. ATPase activity of the control (●) and the DOC-treated (×) microsomes; ADP hydrolysis of the control (○).

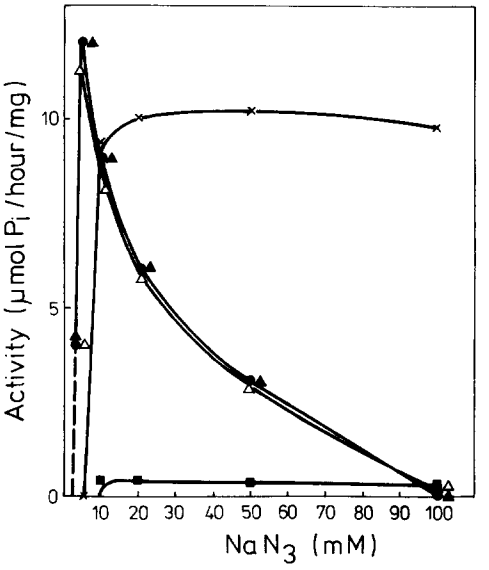


Fig. 2. Effect of azide on the activation of ATPase by monovalent cations in the myometrial microsome fraction. ATPase activities of microsomes isolated in Tris-HCl containing medium and DOC-treated, microsomes were determined as described in Materials and Methods. Activation by the monovalent cations was expressed as a difference between ATPase activities measured in the presence and absence of monovalent cations. In the medium for $Mg^{2+}$-ATPase assay the $Na^+$ concentrations was increased as a function of the added $NaN_3$. In the medium for monovalent cation-activated ATPases the concentration of $Na^+$ was kept constant (100 mM). ATPase activities in the presence of 100 mM Na; (△); 100 mM Na+20 mM $K^+$ (▲) in the control and in the DOC-treated (×) microsomes. Ouabain sensitivity of the monovalent cation-activated ATPase activities of the control (●) and that of the DOC-treated (■) microsomes was tested at 1 mM ouabain.

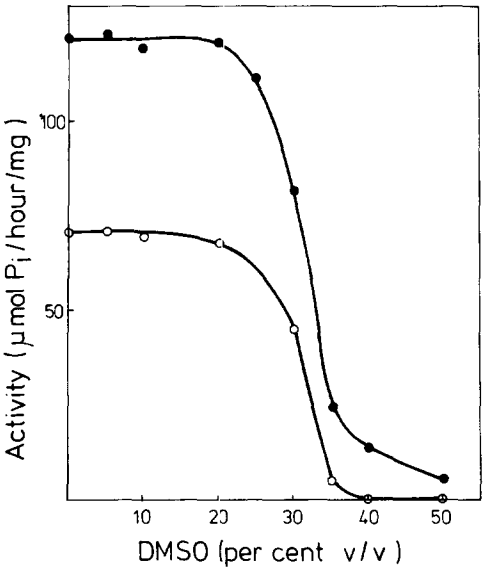


Fig. 3. Inhibition of the $Mg^{2+}$ dependent ATP- and ADP-hydrolysis by DMSO in the myometrial microsome fraction. 1 mg/ml microsomal protein was treated with various concentrations of DMSO as described in Materials and Methods. 25-μl aliquots were transferred to the 1 ml assay mixtures containing ATP (●) or ADP (○), for measuring the enzymatic hydrolysis.

$+ K^+$)-activated ATP hydrolysis could also be measured under certain conditions (Fig. 2). In the control microsomes (not treated with detergent) the monovalent cation activation of ATP hydrolysis reached its highest value at 5 mM $NaN_3$ but it proved ouabain insensitive and almost the same ATPase activity was measured with and without 20 mM $K^+$. The decrease and disappearance of the monovalent cation activation at higher $NaN_3$ concentrations may not be a real effect, because in the medium for the $Mg^{2+}$-ATPase activity assay the $Na^+$ concentration was increased as a function of the added $NaN_3$. On the other hand the ATPase activity of the DOC-treated preparation in the presence of $NaN_3$ could be enhanced by $Na^+ + K^+$ and stayed constant even at a $NaN_3$ concentration as high as 100 mM. Furthermore this activity proved completely ouabain sensitive.

As shown in Fig. 3 the ATP as well as ADP hydrolysis in the presence of $Mg^{2+}$ was found to be inhibited by DMSO. ATP or ADP cleavage by the microsome fraction pretreated with 35–40% DMSO was depressed to below 20% of the control.

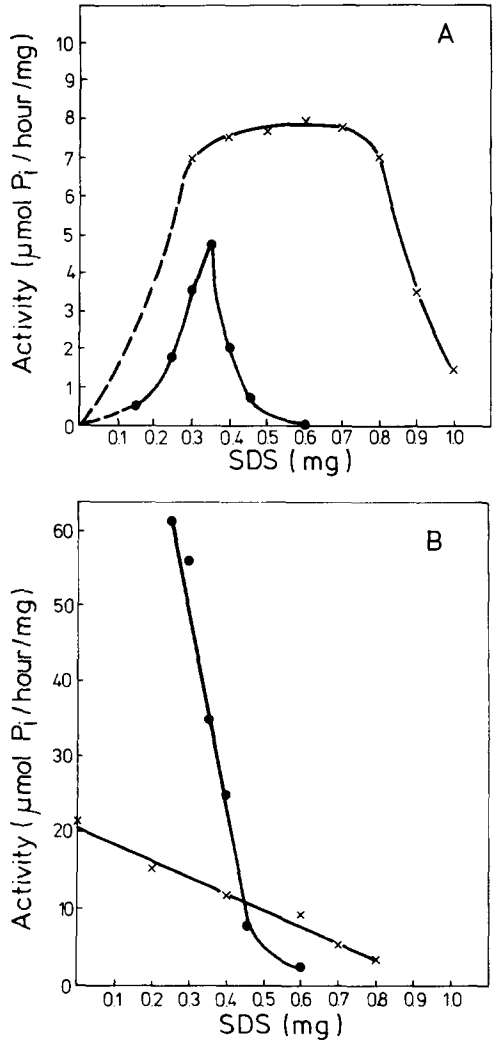


Fig. 4. Effect of SDS and DMSO + SDS treatment of the myometrial microsome fraction on the activity of $Mg^{2+}$ and $(Na^+ + K^+)$-ATPase. 1 mg portions of microsomal protein in 1 ml were treated with various concentrations of SDS (●). (×) indicates when SDS treatment followed a previous incubation with 40% DMSO see Materials and Methods. $(Na^+ + K^+)$-ATPase (A), $Mg^{2+}$-ATPase (B) activities of 25-μl aliquots were determined.

DMSO enhanced the maximum $(Na^+ + K^+)$-ATPase activity developed by SDS treatment (Fig. 4A). In the absence of DMSO an abrupt inactivation of the enzyme occurred as the SDS concentration was increased. Determination of the $(Na^+ + K^+)$-ATPase activity was only possible within a narrow range of SDS concentration without DMSO, where the $Mg^{2+}$-ATPase activity was

about 30–40% of the control. In the presence of DMSO not only was a greater $(Na^+ + K^+)$-ATPase activity obtained, but it was also resistant to higher SDS concentrations, at which the $Mg^{2+}$-ATPase activity did not interfere any more (Fig. 4B). 1 mM ouabain completely inhibited the activation caused by $Na^+ + K^+$.

In conclusion the combined treatment of the myometrial microsomes with DMSO-SDS provided favourable conditions for the manifestation of the $(Na^+ + K^+)$-ATPase activity.

*$Ca^{2+}$ sensitivity of the $(Na^+ + K^+)$-ATPase activity*

The $(Na^+ + K^+)$-ATPase activity manifested in the course of SDS-treatment of the microsomes could be inhibited by 10–100 $\mu$M $Ca^{2+}$. 50 $\mu$M $Ca^{2+}$ caused nearly 50% inhibition (see Table II). In consequence of the detergent treatment about half of the microsomal proteins was solubilized and could be collected in the supernatant (S-Sn) after centrifugation at $100\,000 \times g$ for 60 min. The soluble fraction had no ATPase activity. The $(Na^+ + K^+)$-ATPase activity was completely recovered in the sedimented membrane fragments (S-P). The $Ca^{2+}$-sensitivity substantially changed due to the removal of the solubilized components. Not more than 7–12% inhibition occurred at 50 $\mu$M $Ca^{2+}$ in the pellet in place of the previous 50% decrease in activity. However, the total inhibitory effect observed in the unfractionated preparation could be restored by combining the S-P and S-Sn fraction. On the other hand, the loss of $Ca^{2+}$ sensitivity did not take place when SDS/DMSO-treated microsomes were subjected to the same separation. The amount of solubilized proteins was about the same (data not shown), but the $(Na^+ + K^+)$-ATPase activity of the pellet (SD-P) retained its $Ca^{2+}$ sensitivity (Table IIB).

$Ca^{2+}$ sensitivity of $(Na^+ + K^+)$-ATPase activity in the pellets derived from SDS-treated and DMSO/SDS-treated microsomes (S-P and DS-P)

TABLE II

$Ca^{2+}$ SENSITIVITY OF THE $(Na^+ + K^+)$-ATPase ACTIVITY IN SDS AND DMSO/SDS-TREATED MICROSOMES AND SUBFRACTIONS

An aliquot of the microsomal fraction was treated with SDS and DMSO + SDS. The treated microsomes were separated into a supernatant and pellet by centrifugation at $100\,000 \times g$ for 60 min. The pellets were suspended in the original volume with the homogenization buffer. $(Na^+ + K^+)$-ATPase activity and its $Ca^{2+}$ sensitivity were determined in the SDS- or DMSO/SDS-treated microsomes, furthermore in the supernatants (S-Sn and DS-Sn) as well as the pellets (S-P and DS-P) from the treated microsomes and finally after combining the solubilized and sedimented fractions. ATPase activity was assayed in 25 $\mu$l of each sample. (A) 1 ml microsomes containing 1 mg protein was preincubated with 0.35 mg SDS. (B) 1 mg microsomal protein was preincubated with 40% (v/v) DMSO in 1 ml volume and then treated with 0.7 mg SDS.

| Condition of treatment | $(Na^+ + K^+)$-ATPase activity (mean ± S.D.) (nmol $P_i$/30 min per ml) | | |
|---|---|---|---|
| | + 100 $\mu$M EGTA | + 50 $\mu$M $Ca^{2+}$ | Percentage of $Ca^{2+}$ inhibition |
| (A) Microsomes in SDS | 169 ± 14 | 92 ± 14 | 46 ($n$ = 14) |
| 100 000 × $g$ pellet (S-P) | 195 ± 24 | 183 ± 29 | 7 ($n$ = 9) |
| 100 000 × $g$ supernatant (S-Sn) | 0 | 0 | ($n$ = 4) |
| S-P + S-Sn | 208 ± 48 | 124 ± 38 | 41 ($n$ = 9) |
| (B) Microsomes in DMSO + SDS | 340 ± 20 | 220 ± 20 | 35 ($n$ = 8) |
| 100 000 × $g$ pellet (DS-P) | 442 ± 82 | 236 ± 53 | 47 ($n$ = 16) |
| 100 000 × $g$ supernatant (DS-Sn) | 0 | 0 | ($n$ = 4) |
| DS-P + DS-Sn | 448 ± 62 | 247 ± 47 | 45 ($n$ = 16) |

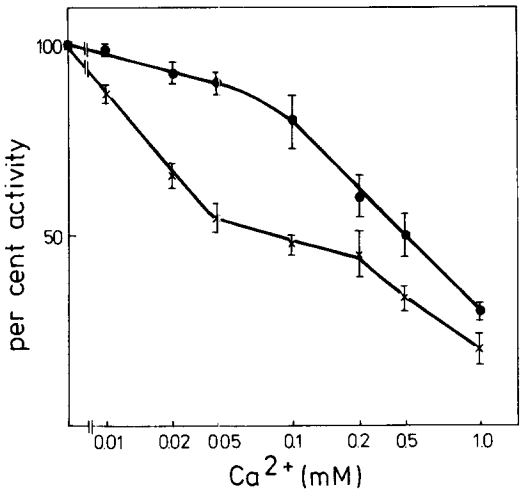


Fig. 5. Effect of $Ca^{2+}$ on the $(Na^+ + K^+)$-ATPase activities of the 100000× *g* pellets derived from SDS (S-P) and SDS/DMSO (DS-P)-treated microsomes. 0.35 SDS was added to 1 mg microsomes (●) or 0.7 mg SDS to the same amount of 40% DMSO pretreated (×) microsomes in 1 ml volume. $(Na^+ + K^+)$-ATPase activities in 25 μg protein of the S-P (spec. act. of S-P: 9.76 μmol $P_i$/h per mg; S.D. 1.22) and DS-P (spec. act. of DS-P: 22.39 μmol $P_i$/h per mg; S.D. 3.12) fractions were measured in the presence of various concentrations of $Ca^{2+}$. $Ca^{2+}$ concentrations less than 150 μM were set using $Ca^{2+}$-EGTA buffer system (see Materials and Methods). 100% $(Na^+ + K^+)$-ATPase activity was measured in the presence of 100 μM EGTA without $Ca^{2+}$ addition.

was compared in Fig. 5 as a function of $Ca^{2+}$ concentration. While in DMSO/SDS-treated preparation (DS-P) the inhibition by $Ca^{2+}$ was pronounced at very low $Ca^{2+}$ concentrations in the SDS-treated fraction (S-P) $Ca^{2+}$ proved ineffective at concentrations of 10–100 μM and became effective only at higher concentrations.

## Discussion

$(Na^+ + K^+)$-ATPase, the generally acknowledged marker enzyme of the plasma membrane has not previously been well characterized in myometrial plasma membrane preparations. This may was due to the interference caused by their high $Mg^{2+}$-ATPase activity and also to the low $Na^+$ and $K^+$ activation and lack of ouabain sensitivity. For this reason enzymatic characterizations of the myometrial plasmamembranes were reduced to determination of the 5′-nucleotidase activity [2,3]. In this study we have developed a feasible method for determination of the $(Na^+ + K^+)$-ATPase activity in the myometrial plasma membrane fraction. This method made it possible to characterize the enzyme itself. So far, we investigated the $Ca^{2+}$ sensitivity of the myometrial $(Na^+ + K^+)$-ATPase activity and attempted to clarify the mechanism of the effect of $Ca^{2+}$.

As far as the $Mg^{2+}$ dependent ATP hydrolysis of the rat myometrial microsome fraction is concerned we have found that for the most part it is due to ATP diphosphohydrolase activity. This enzyme has been reported in the plasma membranes of a number of tissues and demonstrated to be an ectoenzyme, the active center of which and therefore the ATP and ADP hydrolysis are located on the external surface of the membrane [10]. The physiological role of this enzyme is not clear. According to some authors it may regulate the ATP and ADP level in the neighbourhood of the membrane [35]. Others suppose a regulatory role of the enzyme in processes dependent on adenosine and adenine nucleotides such as neurotransmission, blood coagulation, vasodilatation [36]. $NaN_3$ has been accepted to be an inhibitor of the ATP diphosphohydrolase enzyme, applied at millimolar concentrations [10–12]. Our results show that besides $NaN_3$, DMSO can serve as a potent inhibitor of the enzyme.

DMSO is known to reversibly inhibit $(Na^+ + K^+)$-ATPase activity and at a concentration of 40–50%, it causes 100% inhibition. The total $(Na^+ + K^+)$-ATPase activity can be regained by the removal of the reagent [37]. At the same time DMSO found to stimulate *p*-nitrophenylphosphatase activity of the enzyme [38]. for the explanation of the dual effect of DMSO it has been suggested that the compound stabilizes the enzyme-nucleotide complex. This may cause inhibition of the $(Na^+ + K^+)$-ATPase activity. However, DMSO increases the accessibility of the phosphatase site to the exogenous substrate *p*-nitrophenyl phosphate, causing activation of $K^+$-*p*-nitrophenylphosphatase [39]. Preincubation with 35–40% DMSO followed by dilution in the reaction mixture to 1.75–2.0% caused an 8–10% activation of the enzyme.

We found that DMSO proved adventageous to the process of manifestation of the $(Na^+ + K^+)$-ATPase activity by SDS treatment. A much higher

$(Na^+ + K^+)$-ATPase activity could be determined in a wide range of SDS concentrations provided that DMSO was used in preincubation. The reagent seemed to protect the enzyme against inactivation by the detergent.

In order to overcome the inactivation caused by SDS, Forbush et al. [19] carried out detergent treatment in the presence of bovine serum albumin using 100-times more bovine serum albumin than the amount of membrane proteins present. Bovine serum albumin was thought to protect the enzyme by binding some SDS. Bovine serum albumin, however, has no effect on $Mg^{2+}$-ATPase activity. $NaN_3$ which inhibits the $Mg^{2+}$-ATPase, however, does not stabilize $(Na^+ + K^+)$-ATPase manifested by detergent treatment. DMSO offers the combination of the beneficial effects of the two above reagents by lowering $Mg^{2+}$-ATPase activity and at the same time protecting the enzyme from detergent inactivation. In this way a 250% activation by $(Na^+ + K^+)$ can be obtained.

Our results suggest that the $Ca^{2+}$ content in the membrane environment may influence the $(Na^+ + K^+)$-ATPase activity of the smooth muscle plasma membrane fraction. This can also be inferred from the results of other authors too. For example, Allen reported that ouabain-sensitive ATPase activity in dog aorta could be determined only in the presence of SDS + EGTA [40]. Preiss et al. [28] described a fairly low (1 $\mu$mol $P_i$/mg per h) $(Na^+ + K^+)$-ATPase activity in the membrane fraction isolated from sheep artery carotis which could be stimulated by EGTA by 50%. In our experiments the effect of $Ca^{2+}$ in rat myometrial microsome fraction was very characteristic. 10–100 $\mu$M $Ca^{2+}$ causes the same inhibitory effect in myometrium as millimolar $Ca^{2+}$ does in various other tissues. The inhibition that reaches even 50% at less than 100 $\mu$M $Ca^{2+}$, is presumably the consequence of a more complex mechanism. The fact that removal of the components solubilized by a detergent results in the almost complete disappearance of the low concentration-$Ca^{2+}$ sensistivity and also that it may be restored by combining the solubilized and particular fractions allows us to conclude that the process may be mediated by a factor removed from the membrane by SDS. DMSO prevents solubilization of this factor.

The hypothesis that $Ca^{2+}$-dependent processes mediated by some membrane components may influence the $(Na^+ + K^+)$-ATPase activity gains support from the results of Zachowsky et al. too [41]. These authors demonstrated that the maximum inhibition of $(Na^+ + K^+)$-ATPase activity by ouabain could be achieved at orders of magnitude lower concentrations of the reagent if inside-out vesicles obtained frm plasmocytoma cells had been washed by an EGTA-containing buffer. They also showed that several proteins were solubilized by EGTA from the membrane, the readdition of which restored the control ouabain sensitivity in the presence of 100 $\mu$M $Ca^{2+}$. On the basis of our results it can be suggested that the actual capacity of the $(Na^+ + K^+)$-ATPase may be influenced by $Ca^{2+}$-dependent processes.

To test the validity of this hypothesis requires the isolation and characterisation of the solubilized factor(s) from plasma membrane. This is in progress.

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