Effect of ovarian steroids on membrane ATPase activities in microsomes (microsomal fractions) from rat myometrium

Inhibition of a component of the Mg^{2+} -activated ATPase by Ca^{2+} -calmodulin and by oxytocin

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The activities of Mg^{2+} -ATPase (Mg^{2+} -activated ATPase), $(Ca^{2+} + Mg^{2+})$ -activated ATPase and $(Na^{+} + K^{+})$ -activated ATPase have been determined in microsomes (microsomal fractions) obtained from rat myometrium under different hormonal conditions. Animals were either ovariectomized and treated for a prolonged period of time with 17β -oestradiol or progesterone, or myometria were obtained at day 21 of pregnancy. In each case the endometrium was carefully removed. The Mg^{2+} -ATPase consists of two components: an inactivating labile component and a second constant component. The rate of ATP hydrolysis by the labile component of the Mg^{2+} -ATPase declines exponentially as a function of time after adding the membranes to the assay medium; this inactivation is caused by the presence of ATP in the medium. This ATPase activity inhibited by ATP is catalysed by a labile enzyme and hence it gradually diminishes within a few hours, even when the microsomes are kept on ice. This labile component has the highest activity in microsomes from pregnant rats, a lower activity in progesterone-treated rats, and the lowest in 17β -oestradiol-treated rats. This component of the Mg^{2+} -ATPase is not affected by 90 nm-oxytocin. The constant component of the Mg^{2+} -ATPase must be ascribed to a different enzyme, which, in contrast with the labile component, is very stable and not affected by the hormonal status of the animal. This constant component of the Mg^{2+} -ATPase is inhibited both by Ca^{2+} -calmodulin, and by oxytocin in microsomes from pregnant and from progesterone-treated animals, whereas such inhibition does not occur in microsomes from 17β -oestradiol-treated animals. The activity of the $(Na^{+} + K^{+})$ -activated ATPase is not dependent on the hormonal status of the animal. Myometrial microsomes present an ATP-dependent Ca^{2+} transport, irrespective of the hormonal condition, but only in microsomes obtained from rats treated with 17β -oestradiol, can a $(Ca^{2+} + Mg^{2+})$ -activated ATPase activity be demonstrated. This activity can be stimulated by calmodulin.

INTRODUCTION

Plasma membranes from smooth-muscle cells show, in common with all other cells, high levels of a basal non-specific Mg^{2+} -ATPase activity, with K_m values for Mg^{2+} -ATP and Ca^{2+} -ATP in the millimolar range (Kwan, 1982a,b; De Schutter, 1984). Beeler *et al.* (1985) compared the Mg^{2+} -ATPase activities in the microsomes (microsomal fractions) from various rat tissues and found that, in all preparations, the rate of ATP hydrolysis by this Mg^{2+} -ATPase was variable with respect to time, owing to an ATP-dependent inactivation of the enzyme. Microsomes from smooth-muscle cells also present $(Na^{+} + K^{+})$ -ATPase and $(Ca^{2+} + Mg^{2+})$ -ATPase activities, which are assumed to be linked to the $Na^{+} + K^{+}$ pump and the Ca^{2+} pumps respectively. Recently, evidence has been brought forward in favour of the hypothesis that oxytocin inhibits the Ca^{2+} -extrusion mechanism of myometrial plasma membranes (Casteels *et al.*, 1985). This action could be related to the proposed inhibition of the $(Ca^{2+} + Mg^{2+})$ -ATPase by oxytocin (Akerman & Wikström, 1979; Soloff & Sweet, 1982; Popescu *et al.*, 1985).

The aim of the present work was to characterize further the different ATPase activities associated with the myometrial membranes and to investigate the influence of the ovarian steroids on these activities. We have, furthermore, paid special attention to the effects of the addition *in vitro* of oxytocin on the Mg^{2+} -ATPase activity. We were able to establish the presence, in myometrial membranes, of a component of the Mg^{2+} -ATPase activity different from that described by Beeler *et al.* (1985) and which can be inhibited by Ca^{2+} -calmodulin and by oxytocin. Because this inhibition of the Mg^{2+} -ATPase activity by oxytocin might interfere with the assay of the Ca^{2+} -transporting ATPase, a critical reassessment needed to be made of the reported inhibitory effects of oxytocin on the myometrial Ca^{2+} -transporting ATPase [$(Ca^{2+} + Mg^{2+})$ -ATPase] activity.

EXPERIMENTAL

Animals

Female Wistar rats, 9–13 weeks old and weighing approx. 200 g, were used. They were kept under standard

Abbreviations used: Mg^{2+} -ATPase, Mg^{2+} -activated ATPase; $(Ca^{2+} + Mg^{2+})$ -ATPase, $(Ca^{2+} + Mg^{2+})$ -activated ATPase; $(Na^{+} + K^{+})$ -ATPase, $(Na^{+} + K^{+})$ -activated ATPase; p[NH]ppG, guanosine 5'-[β -imidol]triphosphate.

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lighting conditions (from 06.00 until 20.00 h exposed to light and from 20.00 until 06.00 h in the dark). Food and water were available *ad libitum*. Rats were subjected to bilateral ovariectomy by ventral laparotomy after anaesthesia by intraperitoneal injection of 0.20 ml of pentobarbital (60 mg/ml; Nembutal). At the same time, a piece of Silastic tubing (Dow Corning, Midland, MI, U.S.A.), 1.5 cm long, with an internal diameter of 1.6 mm and an external diameter of 2.4 mm and sealed at both ends with Dow Corning medical adhesive silicone type A, was implanted subcutaneously along the spine. The tube was either empty, or contained 30 mg of progesterone or 3 mg of 17β -oestradiol. The resulting plasma concentrations of 17β -oestradiol amounted to 432 ± 59 pg/ml ($n = 5$) and that of progesterone to 13.7 ± 2.0 ng/ml ($n = 5$), as assayed by standard radioimmunoassay techniques. The uteri of these animals were isolated after 2 weeks of hormone treatment. Myometria of pregnant animals were obtained at 14.00 h local time on the day before the expected delivery.

Preparation of the microsomes

The uterine horns were kept in a warmed and oxygenated modified Krebs solution of the following composition: 135.5 mM-NaCl, 5.9 mM-KCl, 1.5 mM- CaCl_2 , 1.2 mM- MgCl_2 , 11.5 mM-glucose, 11.6 mM-Hepes (pH 7.3). They were cut longitudinally in strips 1 mm wide under a dissection microscope. The endometrium was then removed by separating the longitudinal smooth-muscle layer from the circular smooth muscle and the endometrium. This procedure required about 1 h for each uterus. The myometria were frozen in liquid N_2 and kept at -85°C until used.

The myometrial strips were minced with a pair of scissors and homogenized at 0°C with an Ultra-Turrax (Janke and Kunkel, Staufen, Germany) fitted with an N10 shaft and run at maximal speed (twice, 20 s). The homogenization was performed in a Sorvall SS34 polycarbonate tube in a volume corresponding to five times the weight of the tissue and containing 100 mM-KCl, 5 mM- MgCl_2 , 50 mM-Tris/HCl, pH 7.2. A crude vesicular fraction was prepared from the homogenate by centrifugation in a Sorvall SS34 rotor at $1000 g_{\text{max}}$ for 10 min. The supernatant was further centrifuged in a Sorvall SM24 rotor at $10500 g_{\text{max}}$ for 10 min and the pellet discarded. The remaining supernatant was then centrifuged in a Beckman Ti75 rotor at $224000 g_{\text{max}}$ for 30 min and the pellet resuspended in 0.25 M-sucrose. This whole procedure lasted only 90 min.

Enzyme assays

The ATPase activities were measured at 37°C by using an NADH-coupled enzyme system (Wuytack & Casteels, 1980). The Mg^{2+} -ATPase and $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase activities were determined in 1 ml of a medium containing 100 mM-KCl, 30 mM-imidazole/HCl, pH 6.8, 5.7 mM- MgCl_2 , 5 mM-ATP, 1 mM-EGTA, 1.5 mM-phosphoenolpyruvate, 0.26 mM-NADH, 5 mM- NaN_3 , 0.1 mM-ouabain and 40 units of pyruvate kinase and lactate dehydrogenase. Between 10 and 30 μg of microsomal protein was used, and 0.2 mg of saponin was added to unmask latent enzyme activity. The decrease in A_{340} was determined with an Aminco DW-2a spectrophotometer. First the Mg^{2+} -ATPase activity was measured by determining the

rate of ATP hydrolysis in the medium described above. The Mg^{2+} -ATPase activity was calculated from the decrease in A_{340} . In the present paper no correction was made for the 'artefactual' overestimation of the constant component of the Mg^{2+} -ATPase activity caused by the presence in the membranes of adenylate kinase (Missiaen *et al.*, 1988). The $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase activity was measured subsequently by comparing the rate of ATP hydrolysis in the presence of 1 mM-EGTA without added Ca^{2+} and that obtained after addition of 0.87 mM- CaCl_2 (corresponding to a $[\text{Ca}^{2+}]$ of 10^{-5} M). Calmodulin was added at a saturating concentration of 0.6 μM . Calmodulin was prepared from bovine brain as described by Gopalakrishna & Anderson (1982). The effect of oxytocin on the Mg^{2+} -ATPase was determined by comparing the rate of ATP hydrolysis by the Mg^{2+} -ATPase before the addition of oxytocin with that obtained after adding 90 nM-oxytocin. The $(\text{Na}^+ + \text{K}^+)$ -ATPase activity was determined from the decline of the rate of ATP hydrolysis by the constant component of the Mg^{2+} -ATPase (which was measured in a medium without ouabain) induced by adding 10^{-4} M-ouabain. Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Ca^{2+} uptake

Ca^{2+} uptake was measured at 37°C in 1 ml of a solution similar to that used for the determination of $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase activity, except that the coupled enzyme system was omitted (Wuytack *et al.*, 1978). The reaction was initiated by adding 0.87 mM- $^{45}\text{CaCl}_2$ ($[\text{Ca}^{2+}] = 10^{-5}$ M) to the medium. The vesicles were separated from the medium by Millipore filtration. After rinsing and drying the filters, the remaining radioactivity was counted.

Statistical analysis

The results are presented as means $\pm$ S.E.M. The statistical significance of differences between the means was assessed by using Student's *t* test for unpaired data. *P* values of less than 0.05 were considered to be statistically significant.

Materials

Progesterone was obtained from Serva Feinbiochemica (Heidelberg, Germany), 17β -oestradiol and oxytocin from Sigma Chemical Company (St. Louis, MO, U.S.A.), ouabain and saponin from E. Merck (Darmstadt, Germany), and p[NH]ppG from Boehringer (Mannheim, Germany).

RESULTS

ATPase activities in a crude microsomal fraction from myometrium

The activities of the four different ATPases which have been studied in rat myometrial microsomes obtained from animals under different hormonal conditions are illustrated in Table 1. All rats, except the pregnant animals, were ovariectomized and subsequently treated for 2 weeks with oestrogen or progesterone. The myometrial microsomes obtained by our experimental procedure contained both plasma-membrane and endoplasmic-reticulum elements. No attempt was made in the

Table 1. Effect of hormonal treatment on the specific activities (nmol of P_i/min per mg) of the labile Mg²⁺-ATPase, the constant Mg²⁺-ATPase, the (Na⁺ + K⁺)-ATPase and the (Ca²⁺ + Mg²⁺)-ATPase in myometrial membranes

The activity of the labile component of the Mg²⁺-ATPase inactivated by ATP was determined from the slope of the curve representing the ATP hydrolysis as a function of time, 20 s after adding the microsomes to an assay medium containing 20 µg of concanavalin A/ml by subtracting of the slope of the constant component of the Mg²⁺-ATPase. This constant component was determined after the labile component had disappeared completely, i.e. when the ATP hydrolysis became linear as a function of time. The (Na⁺ + K⁺)-ATPase activity was determined as stated in the Experimental section. The (Ca²⁺ + Mg²⁺)-ATPase refers to the change of the Mg²⁺-ATPase activity induced by adding 10⁻⁵ M-Ca²⁺; ' + ' indicates an increase and ' - ' a decrease of the Mg²⁺-ATPase activity. Results are means ± s.e.m. for the numbers of observations given in parentheses. The activities were determined within the first hour after resuspending the membranes in 0.25 M-sucrose.

Enzyme	Hormonal status . . .	Specific activity (nmol/min per mg of protein)		
		17β-Oestradiol	Pregnant	Progesterone
Labile Mg ²⁺ -ATPase		229 ± 30 (11)	3211 ± 203 (9)	2108 ± 103 (6)
Constant Mg ²⁺ -ATPase		276 ± 26 (11)	338 ± 23 (9)	346 ± 31 (6)
(Ca ²⁺ + Mg ²⁺)-ATPase		+66 ± 4 (10)	-54 ± 10 (8)	-67 ± 12 (5)
(Na ⁺ + K ⁺)-ATPase		97 ± 10 (3)	82 ± 12 (5)	101 ± 18 (5)

present study to separate these two types of membranes. This separation procedure takes much longer and would be accompanied by an appreciable decrease of the labile component of the Mg²⁺-ATPase activity, as discussed below.

(1) Mg²⁺-ATPase activity. Fig. 1 illustrates the time-dependency of the Mg²⁺-ATPase activity in myometrial microsomes from animals in different hormonal states. The records on the left of the Figure show the ATP hydrolysis as a function of time after adding the membranes to the assay medium, whereas the graphs on the right represent the rate of ATP hydrolysis by the Mg²⁺-ATPase as a function of time. The rate of ATP hydrolysis by the Mg²⁺-ATPase is not constant; its initial high value decreases exponentially towards a constant value. This finding suggests that the Mg²⁺-ATPase is composed of two components. One component hydrolyses ATP at a constant rate as a function of time. The other component of the Mg²⁺-ATPase hydrolyses ATP at a rate that declines exponentially as a function of time after adding the membranes to the assay medium. This declining activity seems to be due to an ATP-dependent inactivation of the enzyme, as has been described for several rat tissues by Beeler *et al.* (1985). Preincubating the microsomes with 5 mM-Mg²⁺-ATP for 30 min at 37 °C results in a complete disappearance of the time-dependent decline in ATPase activity. After this exposure to ATP, the ATPase activity measured by adding the coupled enzyme system to the cuvette remains constant. The calculated values of the two components of the Mg²⁺-ATPase are given in Table 1. Addition of concanavalin A allows a more accurate estimation of the initial rate of the Mg²⁺-ATPase, because it almost completely blocks the ATP-dependent inactivation (Beeler *et al.*, 1985). We have therefore measured the initial rate of ATP hydrolysis by the labile component of the Mg²⁺-ATPase 20 s after adding the membranes to the medium in the presence of concanavalin A (20 µg/ml). The activity of the labile component of the Mg²⁺-ATPase is strongly dependent on the hormonal condition. It is poorly developed in myometrial microsomes from 17β-oestradiol-pretreated animals, but it is high in microsomes from progesterone-

treated and from pregnant animals. In contrast with the labile component of the Mg²⁺-ATPase activity, the activity of the constant component of the Mg²⁺-ATPase is similar in the myometrial microsomes from oestrogen-treated, from progesterone-treated and from pregnant rats. It is at least 3–4 times higher than that observed in pig antral smooth muscle (De Schutter *et al.*, 1984).

The rate of ATP hydrolysis by the constant component and by the labile component of the Mg²⁺-ATPase is illustrated in Fig. 2 for microsomes from pregnant myometrium that have been stored on ice for different periods of time. The activity of the constant component does not decrease by keeping the microsomes on ice for 6 h, whereas the labile component declines appreciably. As a consequence, the typical non-linearity of ATP hydrolysis gradually disappears during storage of the microsomes (Fig. 3). This time-dependent decline in the labile Mg²⁺-ATPase activity is most pronounced in microsomes from pregnant animals.

The two components of the Mg²⁺-ATPase activity are differently affected by oxytocin. The addition of 90 nM-oxytocin to myometrial microsomes from 17β-oestradiol-pretreated rats does not affect the activity of the constant component, whereas it reduces this activity in microsomes from pregnant and progesterone-treated animals by 41.16 ± 5.39 (*n* = 9) and 21.08 ± 3.11 (*n* = 6) nmol of P_i/min per mg of protein respectively. p[NH]ppG at 100 µM induces the same degree of inhibition and prevents the effect of oxytocin. It is therefore likely that this inhibitory effect is mediated by a G-protein. The observation that the addition of oxytocin inhibits the Mg²⁺-ATPase in microsomes from pregnant myometrium only after a delay of 2.31 ± 0.13 (*n* = 5) min suggests that more than one step is involved in the inhibition of the constant component of the Mg²⁺-ATPase activity by oxytocin.

In contrast with this marked effect of oxytocin on the constant component of the Mg²⁺-ATPase, no effect of this substance on the labile Mg²⁺-ATPase activity could be established. Addition of microsomes to a medium containing oxytocin or 100 µM-p[NH]ppG did not affect the time-dependent inactivation of this Mg²⁺-ATPase. These experiments were performed during the first 1 h after resuspending the microsomal pellet, i.e. at the stage

Fig. 1. ATP hydrolysis and the rate of ATP hydrolysis due to Mg^{2+} -ATPase activity as a function of time in microsomes from rat myometrium in different hormonal conditions

The left-hand side shows the Mg^{2+} -ATPase activity determined during the first hour after the preparation of the membranes. Between 10 and 30 μg of microsomal protein was added at zero time in a cuvette of volume 1 ml. Each of the records is representative for more than 20 experiments on different preparations. On the right the rate of ATP hydrolysis by the Mg^{2+} -ATPase activity is given as a function of time, and has been obtained by differentiating the curve represented in the panel on the left. (a) Was obtained with microsomes from pregnant-rat myometrium, (b) with those from progesterone-pretreated and (c) with microsomes from 17β -oestradiol-pretreated myometrium.

at which the Mg^{2+} -ATPase still exhibits a pronounced non-linearity. The measurements of the control sample and the sample containing either oxytocin or p[NH]ppG were performed simultaneously in order to avoid any interference by the time-dependent inactivation of the Mg^{2+} -ATPase.

(2) ($Ca^{2+} + Mg^{2+}$)-ATPase activity. Increasing $[Ca^{2+}]$ to 10^{-5} M in the incubation medium containing 1 mM- $[Mg^{2+}]$ augments the ATPase activity only in myometrial microsomes of the 17β -oestradiol-treated group (Table 1), and further addition of 0.6 μM -calmodulin increases this activity by a factor of 1.57 ± 0.12 ($n = 10$).

Fig. 2. Constant Mg²⁺-ATPase activity and labile Mg²⁺-ATPase activity in microsomes from pregnant-rat myometrium as a function of time after resuspending the membranes

The constant Mg²⁺-ATPase activity (a) and the labile Mg²⁺-ATPase activity (b) were measured as indicated in the legend to Table 1. These activities were determined after the microsomes had been stored on ice for different times. The time of storage on ice is represented on the abscissa and is expressed in hours. The ordinate represents the Mg²⁺-ATPase expressed as a percentage of the Mg²⁺-ATPase activity obtained immediately after resuspending the membranes in 0.25 M-sucrose (means $\pm$ S.E.M. for the numbers of observations given in parentheses).

In contrast, the rate of ATP hydrolysis by myometrial microsomes from progesterone-treated or pregnant animals is significantly diminished by the same concentrations of [Ca²⁺] and calmodulin [by a factor of 1.65 ± 0.17 ($n = 7$) and of 1.52 ± 0.18 ($n = 5$) respectively]. These effects can be described as an anomalous or a 'negative (Ca²⁺ + Mg²⁺)-ATPase activity' (see the Discussion section).

(3) (Na⁺ + K⁺)-ATPase activity. The specific activity of the (Na⁺ + K⁺)-ATPase (nmol of P_i/min per mg of protein) in myometrial microsomes is not significantly different for the three standardized groups and amounts in each group to about 27% of the 'constant-component' Mg²⁺-ATPase activity (Table 1).

Ca²⁺ uptake by the myometrial microsomes

The Ca²⁺ uptake by the myometrial microsomes prepared from animals in different hormonal conditions is shown in Fig. 4. The ATP-dependent Ca²⁺-transport is enhanced by 5 mM-oxalate, and this enhancing effect is more pronounced in microsomes obtained from 17 β -oestradiol-treated animals than in those from the other groups.

DISCUSSION

Preparation of the membranes

Because one of the goals of the present work was to investigate the effect of ovarian steroids on the membrane ATPases, we have tried to optimize our technique to

Fig. 3. Change of the ATP hydrolysis by the Mg²⁺-ATPase during storage on ice in microsomes from rat myometrium in the three hormonal conditions

The rate of ATP hydrolysis by the Mg²⁺-ATPase was determined at different time intervals after the preparation of the membranes in order to study the effect of storage on ice on the Mg²⁺-ATPase activity. The storage time interval is represented at the right side of the tracing and is expressed in h; '0' corresponds to the moment of resuspending the membranes in 0.25 M-sucrose. Microsomes were obtained from myometrium from pregnant rats (a), from progesterone-pretreated rats (b), or from 17 β -oestradiol-pretreated animals (c). The curves are original tracings, and are representative for at least three measurements on three different membrane preparations.

prime the animals hormonally. Ovariectomy is essential, and the administration of the steroid must be extended over a sufficiently long period of time in order to obtain a reproducible hormonal condition of the animal. The preparation of myometrial microsomes requires a complete removal of the endometrium before homogenization. Although scraping is a rapid technique, it fails to

Fig. 4. Ca^{2+} uptake by myometrial microsomes from rats in different hormonal conditions

The Ca^{2+} uptake is given in nmol/mg of protein as a function of time (min): $\times$, in the absence of ATP; $\circ$, in the presence of 5 mM-ATP; $\bullet$, in the presence of 5 mM-ATP and 5 mM-oxalate. Microsomes were obtained from the myometrium of pregnant rats (a), of progesterone-pretreated rats (b) or of 17β -oestradiol-pretreated animals (c).

give a perfect separation of myometrium and endometrium because the thickness of the endometrium exceeds by far that of the myometrium. We have therefore preferred the time-consuming dissection.

Mg^{2+} -ATPase activity

The Mg^{2+} -ATPase activity in myometrial microsomes consists of two different components: a labile declining component and a constant component. A Mg^{2+} -ATPase activity decreasing as a function of time has been observed in all vascular smooth-muscle membrane preparations which we have studied. Also, Barron &

DiSalvo (1979) and Beeler *et al.* (1983, 1985) observed that microsomes from bovine aorta and plasma-membrane preparations from a wide variety of tissues present a Mg^{2+} -ATPase activity with an initial phase of high activity. The latter authors furthermore observed that concanavalin A prevents this time-dependent decline of the Mg^{2+} -ATPase activity. The same effect of concanavalin A has also been observed in our preparations. This labile Mg^{2+} -ATPase in myometrial membranes could therefore be an enzyme analogous to that described by Beeler *et al.* (1985) and it might also be an ectoenzyme. Its decreasing activity seems also to be due to a progressive inhibition of the ATPase by high concentrations of ATP. An alternative explanation, namely that the initial non-linearity of the curves representing the ATP concentration as a function of time is due to a time-dependent decrease of a contaminating NADH oxidase activity, has been excluded (Missiaen *et al.*, 1988). Our findings also demonstrate that this labile Mg^{2+} -ATPase activity is modulated by the hormonal condition of the animal.

The Mg^{2+} -ATPase activity also exhibits a second component, which hydrolyses ATP at a constant rate. This activity differs from the labile component by its stability during storage, by its not being affected by the hormonal status of the animal, and by its sensitivity to oxytocin and Ca^{2+} -calmodulin in certain hormonal conditions. The function of this component of the Mg^{2+} -ATPase is as yet unknown. An unexpected finding is that the constant component of the Mg^{2+} -ATPase activity in the myometrium of progesterone-treated and of the pregnant rats is inhibited by Ca^{2+} and that this inhibition becomes even more pronounced in the presence of calmodulin. To the best of our knowledge such an inhibitory effect of 10^{-5} M- Ca^{2+} and calmodulin on the ATPase measured in the presence of millimolar Mg^{2+} has not been described either in smooth muscle or in any other tissue. This inhibitory effect is hormonally dependent and does not occur in 17β -oestradiol-pretreated-rat microsomes. The constant component of the Mg^{2+} -ATPase activity in myometrial microsomes from progesterone-treated and pregnant rats is also inhibited by oxytocin, both in the presence and in the absence of Ca^{2+} . An 'artefactual' effect of this hormone on the ATPase assay itself can be excluded, because oxytocin does not affect the Mg^{2+} -ATPase activity in microsomes from 17β -oestradiol-pretreated rats, or from porcine antral smooth muscle. This inhibitory effect of oxytocin cannot be due to an inhibition of a remaining $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase activity, which has been proposed by Soloff & Sweet (1982), Akerman & Wikström (1979) and Popescu *et al.* (1985). Even if our assay system for Mg^{2+} -ATPase were contaminated with a total amount of 0.05 mM- Ca^{2+} , the EGTA/ATP-buffered enzyme assay would decrease the free Ca^{2+} concentration to a value below $0.1 \mu\text{M}$ (Wuytack & Casteels, 1980). The presumed $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase activity would at this low $[\text{Ca}^{2+}]$ be much lower than the component of the ATPase activity inhibited by oxytocin. Moreover, the inhibition by oxytocin does not become larger at Ca^{2+} concentrations that maximally activate $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase. A high-affinity Ca^{2+} -ATPase found in rat liver is also inhibited by a number of agonists, including peptides such as vasopressin (Lin *et al.*, 1983) or glucagon (Lotersztajn *et al.*, 1984), but here also there is some doubt regarding the function of this enzyme as a Ca^{2+} -

transporting ATPase (Lin, 1985). It has to be pointed out that our present findings do not exclude the possibility that, under certain experimental conditions, oxytocin may also affect Ca²⁺ transport.

(Ca²⁺ + Mg²⁺)-ATPase activity

The (Ca²⁺ + Mg²⁺)-ATPase activity of myometrial microsomes also depends on the hormonal status of the animal. Adding 10⁻⁵ M-Ca²⁺ and 0.6 μ M-calmodulin to myometrial microsomes from 17 β -oestradiol-treated animals increases the ATPase activity, whereas both additions cause a diminution in the same ATPase activity in microsomes from progesterone-treated and pregnant animals. The ⁴⁵Ca²⁺-uptake experiments indicate that, irrespective of the hormonal status, the membranes of the microsomes have an ATP-dependent Ca²⁺-transport system that, in microsomes from 17 β -oestradiol-treated animals, is stimulated to a larger extent by oxalate than that in microsomes from animals in other hormonal conditions. This difference might reflect a difference of the proportion of plasma-membranes and endoplasmic-reticulum elements in the microsomes. Such ATP-dependent Ca²⁺ uptake has to be accompanied by a comparable increase of the positive (Ca²⁺ + Mg²⁺)-ATPase activity. However, this increase can be masked by a much larger inhibition of the Mg²⁺-ATPase by Ca²⁺ and Ca²⁺-calmodulin. These interactions may explain the lack of correlation in the present results between the (Ca²⁺ + Mg²⁺)-ATPase activity and ATP-dependent Ca²⁺ transport. The same phenomenon could also be the reason why Barron & DiSalvo (1979) could not establish a (Ca²⁺ + Mg²⁺)-ATPase activity associated with Ca²⁺ transport in smooth-muscle microsomes.

An inhibition of the (Ca²⁺ + Mg²⁺)-ATPase activity by oxytocin has recently been reported in the myometrium of oestradiol-treated rabbits (Akerman & Wikström, 1979), in the myometrium from rats treated with diethylstilboestrol (Soloff & Sweet, 1982) and in human myometrium at term (Popescu *et al.*, 1985). These three groups concluded that oxytocin inhibits almost completely the (Ca²⁺ + Mg²⁺)-ATPase and, also in their opinion, the Ca²⁺-pump activity. It should be pointed out that, in the second study, maximal stimulation of the

(Ca²⁺ + Mg²⁺)-ATPase activity occurred at a free-Ca²⁺ concentration of 60 nM, which is rather unusual for a Ca²⁺-transporting ATPase. Because all assay media for measuring (Ca²⁺ + Mg²⁺)-ATPase contain millimolar Mg²⁺, this (Ca²⁺ + Mg²⁺)-ATPase activity is measured together with the Mg²⁺-ATPase activity. The inhibition of the constant component of the Mg²⁺-ATPase by 90 nM-oxytocin should therefore be taken into account when one investigates inhibition of the (Ca²⁺ + Mg²⁺)-ATPase activity.

REFERENCES

- Akerman, K. E. & Wikström, K. F. (1979) *FEBS Lett.* **97**, 283–287
- Barron, J. T. & DiSalvo, J. (1979) *Proc. Soc. Exp. Biol. Med.* **160**, 259–262
- Beeler, T. J., Gable, K. S. & Keffer, J. M. (1983) *Biochim. Biophys. Acta* **734**, 221–234
- Beeler, T. J., Wang, T., Gable, K. & Lees, S. (1985) *Arch. Biochem. Biophys.* **243**, 644–654
- Casteels, R., Droogmans, G. & Missiaen, L. (1985) *J. Physiol. (London)* **369**, 96P
- De Schutter, G., Wuytack, F., Verbist, J. & Casteels, R. (1984) *Biochim. Biophys. Acta* **773**, 1–10
- Gopalakrishna, A. & Anderson, W. B. (1982) *Biochim. Biophys. Res. Commun.* **104**, 830–836
- Kwan, C. Y. (1982a) *Enzyme* **28**, 317–327
- Kwan, C. Y. & Ramlal, T. (1982b) *Biochem. Int.* **4**, 439–449
- Lin, S.-H. (1985) *J. Biol. Chem.* **260**, 10976–10980
- Lin, S.-H., Wallace, M. A. & Fain, J. N. (1983) *Endocrinology (Baltimore)* **113**, 2268–2275
- Lotersztajn, S., Epond, R. M., Mallat, A. & Pecker, F. (1984) *J. Biol. Chem.* **259**, 8195–8201
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Missiaen, L., Wuytack, F. & Casteels, R. (1988) *Biochem. J.* **250**, 579–588
- Popescu, L. M., Nutu, O., Panoiu, C. (1985) *Biosci. Rep.* **5**, 21–28
- Soloff, S. & Sweet, P. (1982) *J. Biol. Chem.* **257**, 10687–10693
- Wuytack, F. & Casteels, R. (1980) *Biochim. Biophys. Acta* **595**, 257–263
- Wuytack, F., Landon, E., Fleischer, S. & Hardman, J. G. (1978) *Biochim. Biophys. Acta* **540**, 253–269

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