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Elicitation of the brain microsomal (Mg^{2+} + Ca^{2+})-activated ATPase by digitonin treatment

It is well known that brain microsomes possess Mg^{2+}-ATPase (ATP phosphohydrolase, EC 3.6 1.3), insensitive to g-strophanthin, besides (Na^+ + K^+)-ATPase which is completely inhibited by g-strophanthin[1]. In the previous paper[2] it was reported that Mg^{2+}-ATPase of brain microsome was slightly activated by low concentrations of Ca^{2+} such as 10^{-5} M.

Recently NAKAMARU[3] found that this (Mg^{2+} + Ca^{2+})-stimulated ATPase activity, (Mg^{2+} + Ca^{2+})-ATPase, of brain microsome was much higher in the microsomal fraction than in the mitochondrial fraction, suggesting that the (Mg^{2+} + Ca^{2+})-ATPase was located in microsomes but not in mitochondria

In this communication we wish to report considerable elicitation of brain microsomal (Mg^{2+} + Ca^{2+})-ATPase by digitonin treatment, and the differences in sensitivity to digitonin of (Mg^{2+} + Ca^{2+})-ATPase and Mg^{2+}-ATPase, which is insensitive to ethyleneglycol-bis-(β-aminoethylether)-*N*,*N*'-tetraacetate (EGTA)

Brain microsomes were prepared as described previously[2] with the following modifications Pig brain was homogenized for 20 sec with 10 vol of 0 32 M sucrose. After centrifugation at 1500 × *g* for 10 min, the supernatant was centrifuged at 17 000 × *g* for 1 h The resulting supernatant was further centrifuged at 64 000 × *g* for 1 h Then the precipitates were suspended in 5 mM Tris–maleate–NaOH (pH 7 2), and recentrifuged at 64 000 × *g* for 1 h Resulting precipitates were thoroughly homogenized in the above buffer (3–4 mg protein per ml) and used as microsomes

TABLE I

EFFECT OF CATIONS AND EGTA ON THE 0 1% DIGITONIN-TREATED BRAIN MICROSOMAL ATPASE
Conditions for the assay were 50 mM Tris–HCl (pH 7 4), 1 25 mM ATP, at 37°

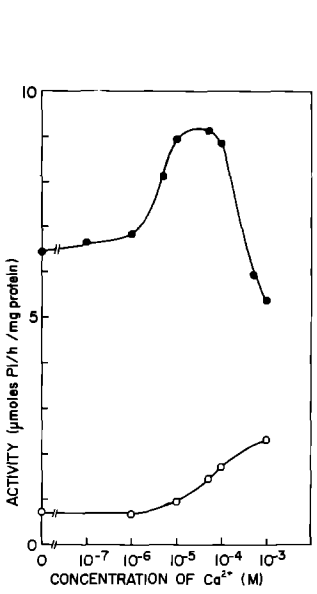
Microsomes	*130 mM Na^+ 20 mM K^+*	*Mg^{2+} (mM)*	*Ca^{2+} (mM)*	*EGTA (mM)*	*Strophanthin (mM)*	*Activity**	*Strophanthin-sensitive ATPase or (Mg^{2+} + Ca^{2+})-ATPase**
Untreated	+	2	0	0	0	13 7	
	+	2	0	0	0 125	4 4	9 3
	−	0	0	0	0 125	0 4	
	−	5	0	0	0 125	4 2	
	−	5	0 03	0	0 125	5 3	
	−	5	0	0 125	0 125	3 5	1 8
Treated with 0 1% digitonin	+	2	0	0	0	27 6	
	+	2	0	0	0 125	7 3	20 3
	−	0	0	0	0 125	0 6	
	−	5	0	0	0 125	7 4	
	−	5	0 03	0	0 125	11 1	
	−	5	0	0 125	0 125	3 9	7 2

* μmoles P_i per h per mg protein

Subsequent treatment of the microsomes with digitonin was, when necessary, carried out by adding an equal volume of a digitonin solution of twice the desired concentration. After keeping for 10 min at room temperature, 0.5 ml of the mixture was submitted to the enzyme assay.

Determination of ATPase activity was performed as described previously[2]. Strophanthin-insensitive ATPase was determined in the presence of Mg^{2+}, Na^+, K^+ and g-strophanthin ($Na^+ + K^+$)-ATPase was calculated as a strophanthin-sensitive activity, a difference between total activity assayed in the presence of Mg^{2+}, Na^+ and K^+ and in the absence of ~~g-strophan~~thin, ~~and the~~ strophanthin-insensitive ATPase activity. ($Mg^{2+} + Ca^{2+}$)-ATPase was calculated as a difference between an activity assayed in the presence of 5 mM Mg^{2+}, $3 \cdot 10^{-5}$ M Ca^{2+} and g-strophanthin, and EGTA-insensitive activity (Ca^{2+}-independent Mg^{2+}-ATPase) assayed in the presence of 5 mM Mg^{2+}, $3 \cdot 10^{-5}$ M Ca^{2+} (or absence), g-strophanthin and EGTA In the case of digitonin-treated microsomes, the concentration of digitonin was reduced to one eighth that used in the treatment, in the 4 ml of reaction medium

Table I shows that ATPase activity in the presence of g-strophanthin is in-



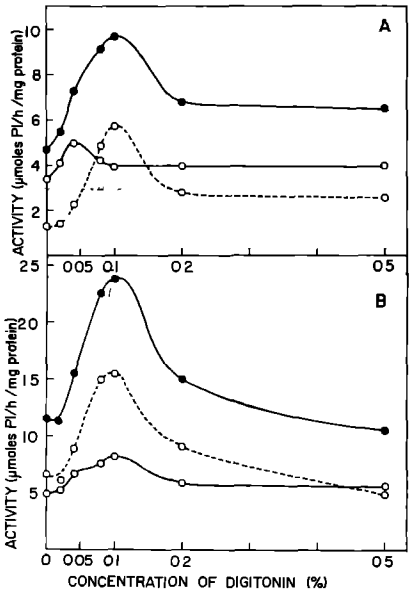


Fig 1. Effect of Ca^{2+} concentration on the brain microsomal strophanthin-insensitive ATPase after 0.1% digitonin treatment Conditions for the assay were 50 mM Tris–HCl (pH 7.4), 0.125 mM g-strophanthin, 1.25 mM ATP, at 37° ●—●, in the presence of 5 mM Mg^{2+}, ○—○, in the absence of Mg^{2+}

Fig 2 Effect of digitonin treatment A On the ($Mg^{2+} + Ca^{2+}$)-ATPase and Ca^{2+}-independent Mg^{2+}-ATPase Conditions for the assay were 50 mM Tris–HCl (pH 7.4), 5 mM Mg^{2+}, $3 \cdot 10^{-5}$ M Ca^{2+}, 0.125 mM g-strophanthin, 1.25 mM ATP, at 37°. ○—○, in the presence of 0.125 mM EGTA (Ca^{2+}-independent Mg^{2+}-ATPase), ●—●, in the absence of EGTA, ○— — —○, difference of above two activities (($Mg^{2+} + Ca^{2+}$)-ATPase) B On the strophanthin-sensitive and -insensitive ATPase ●—●, total activity, ○—○, strophanthin-insensitive ATPase, ○— — —○, strophanthin-sensitive ATPase Conditions were 50 mM Tris–HCl (pH 7.4), 2 mM Mg^{2+}, 130 mM Na^+, 20 mM K^+, 1.25 mM ATP, 0.125 mM g-strophanthin (only in the case of strophanthin-insensitive ATPase), at 37°.

creased by the addition of $3 \cdot 10^{-5}$ M Ca^{2+} and largely inhibited by EGTA, suggesting the presence of (Mg^{2+} + Ca^{2+})-activated ATPase. This (Mg^{2+} + Ca^{2+})-ATPase is remarkably stimulated, as is strophanthin-sensitive ATPase, by subsequent digitonin treatment. After this treatment, strophanthin-sensitive ATPase and (Mg^{2+} + Ca^{2+})-ATPase increase 2-fold and 4-fold, respectively, and the specific activity of (Mg^{2+} + Ca^{2+})-ATPase is about one third that of strophanthin-sensitive ATPase Only low concentrations of Ca^{2+} are effective for the activation of (Mg^{2+} + Ca^{2+})-ATPase and the activity is lowered with an increase of Ca^{2+}, as shown in Fig 1. Maximum activation is obtained with added $3 \cdot 10^{-5}$ M Ca^{2+} in the presence of 5 mM Mg^{2+} A considerable amount of the activity observed without addition of Ca^{2+} is undoubtedly due to some contaminated Ca^{2+}, because EGTA can reduce the activity When Mg^{2+} is not added, the activity is low at low concentrations of Ca^{2+} Gradual increase of the activity with increase of Ca^{2+} is in accordance with results reported previously[2]

There is a difference between EGTA-insensitive Mg^{2+}-ATPase and (Mg^{2+} + Ca^{2+})-ATPase with respect to sensitivity to digitonin As shown in Fig 2A, 0.04% digitonin treatment is the most effective for stimulation of EGTA-insensitive ATPase but the stimulation is slight, whereas 0 1% digitonin considerably stimulates (Mg^{2+} + Ca^{2+})-ATPase Fig 2B shows the effect of digitonin treatment on the strophanthin-sensitive ATPase. It can be seen that 0 1% digitonin is the most effective for the stimulation of the strophanthin-sensitive ATPase These results suggest the similarity of strophanthin-sensitive ATPase to (Mg^{2+} + Ca^{2+})-ATPase rather than EGTA-insensitive Mg^{2+}-ATPase.

Recent demonstration of Ca^{2+} binding of brain microsome induced by ATP[4] may show a resemblance of brain microsome to muscle microsome in its properties of (Mg^{2+} + Ca^{2+})-ATPase[5] and Ca^{2+} binding[6]. In the presence of oxalate and Mg^{2+}, HASSELBACH AND MAKINOSE[7] showed that Ca^{2+} could activate the rate of ATP hydrolysis by muscle microsomes, and suggested that Ca^{2+} transport and the rates of ATP hydrolysis were linked In the brain microsome (Mg^{2+} + Ca^{2+})-ATPase also may have some relationship to Ca^{2+} binding requiring ATP

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