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Stimulation of the phosphatase activity of (Na^+,K^+)-ATPase preparations by ouabain

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SUMMARY

1. We have observed that under certain conditions the phosphatase activity of (Na^+, K^+)-ATPase preparations is stimulated by ouabain and other cardioactive steroids.

2. This effect requires Mg^{2+} and occurs in the absence of Na^+ and K^+ and at sub-optimal substrate concentrations.

3. The maximal stimulation observed was greater than 3-fold.

Studies of the effect of cardioactive glycosides on the (Na^+, K^+)-ATPase (ATP phosphohydrolase, EC 3.6.1.3), which is responsible for the transport of Na^+ and K^+ through membranes, and on its partial reactions has contributed a great deal to our understanding of this process (*e.g.* see Whittam and Wheeler[1]). In this report we wish to draw attention to a previously unrecognised effect of cardioactive glycosides on the enzyme complex.

TABLE I

STIMULATION OF UMBELLIFERONE PHOSPHATASE AND *p*-NITROPHENYLPHOSPHATASE ACTIVITIES BY OUABAIN

Assays were performed with 0.2 mM substrate, 4 mM $MgCl_2$ and 28 μg/ml enzyme★ in 0.08 M Tris–HCl, pH 7.8, at 37° and the activities were determined by the methods previously reported[2,3].

Substrate	*Activity (μmoles product/mg protein per h)*	
	With Mg^{2+}	*+0.4 mM ouabain*
Umbelliferone phosphate	0.12	0.29
p-Nitrophenyl phosphate	0.08	0.19

★(Na^+, K^+)-ATPase prepared from rat brain by the method of Skou[4].

In a previous paper[2] we reported the use of a fluorimetric assay method for the K^+-phosphatase associated with (Na^+, K^+)-ATPase. The method, which employs umbelliferone phosphate as the substrate, is highly sensitive and permits assays to be performed at low substrate concentrations. While using this method to study the inhibition of the K^+-dependent phosphatase activity by ouabain we were surprised to find that the addition of ouabain caused a significant increase in the Mg^{2+}-dependent phosphatase activity. A similar increase was observed with *p*-nitrophenyl phosphate as the substrate under the same conditions (see Table I). It can be seen that ouabain caused an increase of about 140% with each substrate.

The substrate concentration employed in these experiments was somewhat lower than that normally employed in investigations of *p*-nitrophenylphosphatase activity (usually 4 mM). This is significant because the results shown in Fig. 1 indicate that little activation occurs at substrate concentrations greater than 2 mM.

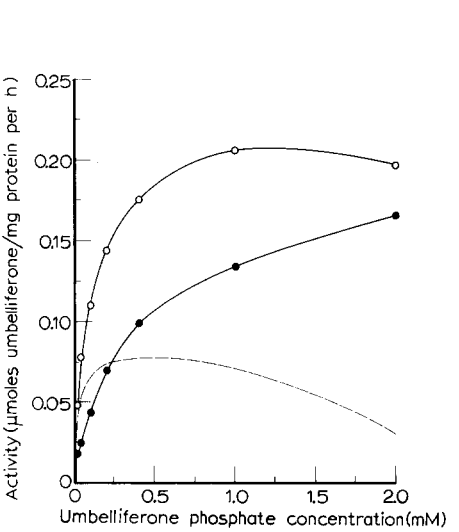


Fig.1. Effect of substrate concentration on the extent of ouabain activation. Assays were conducted with substrate, 4 mM $MgCl_2$ and 14 μg/ml rat brain ATPase. Other details were as given in Table I. •, control; o, with 0.2 mM ouabain. The dotted line indicates the result of substracting the control from the activity in the presence of ouabain.

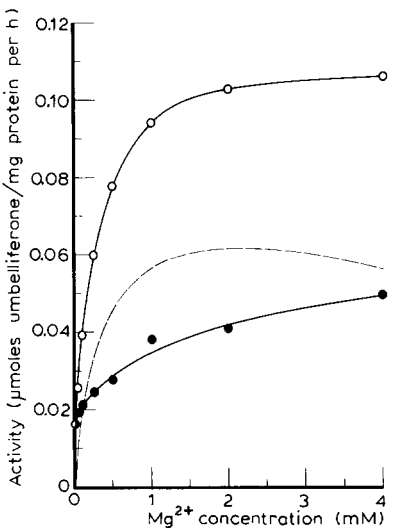


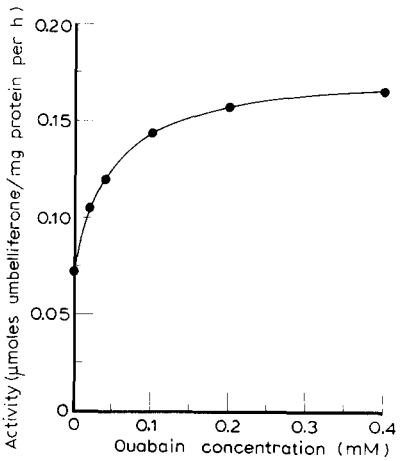
Fig.2. Effect of Mg^{2+} concentration on the extent of activation by ouabain. Assays were conducted with 0.2 mM umbelliferone phosphate and 14 μg/ml rat brain ATPase. Other details were as given in Table I. •, control; o, with 0.2 mM ouabain. The dotted line gives the difference between the activities in the presence and absence of ouabain.

The dependence of the activation effect on the presence of Mg^{2+} is shown in Fig. 2 and the effect of increasing concentrations of ouabain is shown in Fig. 3.

Similar activation was observed with four other cardioactive steroids (digitoxin, digitoxigenin, digoxin and digoxigenin) but two steroids which are not cardioactive (cholesterol and prednisolone) were without effect.

In addition to the preparation from rat brain we also tested preparations from beef brain (prepared by the method of Schoner *et al.*[5]), beef heart (by the method of Besch

et al.[6]) and rat heart (by the method of Akera *et al.*[7]). All these preparations were activated by ouabain but with the two heart preparations it was necessary to use a much higher concentration of ouabain (4 mM) and the extent of activation was much less (about 20%).



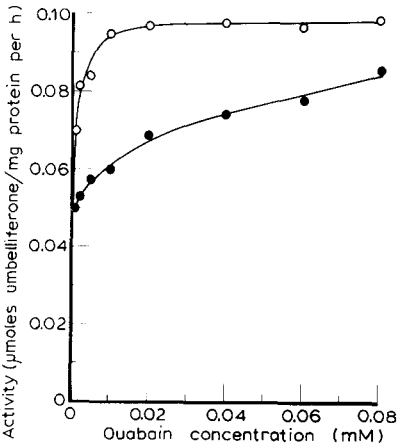


Fig.3. Effect of ouabain concentration. Assays contained 0.2 mM umbelliferone phosphate, 2 mM $MgCl_2$ and 14 μg/ml rat brain ATPase. Other details were as given in Table I.

Fig.4. Effect of ATP at low ouabain concentrations. Assays contained 0.2 mM umbelliferone phosphate, 0.5 mM $MgCl_2$ and 14 μg/ml rat brain ATPase. Other details were as given in Table I. •, control; o, with 4 μM ATP.

With the beef brain preparation on the other hand maximal activation (about 70%) was observed at a ouabain concentration of about 0.04 mM. The reason for these differences between preparations from different tissues is not clear and is the subject of further investigation.

Nagai *et al.*[8] observed that ouabain inhibition of the K^+-phosphatase was competitive with respect to K^+ which suggests that ouabain may bind at the K^+ site. If this is so, the activation by ouabain in the absence of K^+ could be explained by assuming that ouabain exerts a limited activating effect in the same manner as K^+. Now if this is correct it might be expected that at low ouabain concentrations further activation might be observed on the addition of ATP + Na^+ as is observed with low K^+ concentrations[9]. When this was tested we found that activation did indeed occur on the addition of ATP (see Fig. 4) but Na^+ was not required for this effect. This concentration of ATP (4 μM) had no effect when added in the absence of ouabain. The lack of requirement for Na^+ could indicate either that this is an entirely different effect to that observed at low K^+ concentrations or that ouabain exerts a Na^+-like effect in addition to its activating effect at the K^+ site. Some support for this latter suggestion is provided by the observation of Rodnight *et al.*[10] that ouabain stimulates the formation of the phosphorylated intermediate at sub-optimal Na^+ concentrations but inhibits when the Na^+ concentration is optimal. These results would seem to indicate that competition occurs between Na^+ and ouabain at the Na^+ binding site.

Stimulation of the phosphatase activity by ouabain has not previously been observed but there have been several reports of a small stimulation of ATPase activity in the

presence of Na^+ and K^+ by very low concentrations of ouabain (about 10^{-10} M) (see refs. 11–14). A small rather variable stimulation by 10^{-4} M ouabain has also been reported[15] but the conditions under which all these effects were observed were so different to those reported in this paper that it seems unlikely that there is any connection between them. On the other hand, a report by Klein[16] of a small but definite stimulation of the Mg^{2+}-ATPase activity of chick heart by 10^{-3}–10^{-4} M ouabain may be another manifestation of the same phenomenon.

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REFERENCES

1 R. Whittam and K.P. Wheeler, *Ann. Rev. Physiol.*, 32 (1970) 21.
2 B.J.R. Pitts and A. Askari, *Biochim. Biophys. Acta*, 227 (1971) 288.
3 O.A. Bessey, O.H. Lowry and M.J. Brock, *J. Biol. Chem.*, 164 (1946) 321.
4 J.C. Skou, *Biochim. Biophys. Acta*, 58 (1962) 314.
5 W. Schoner, C. Von Ilberg, R. Kramer and W. Seubert, *European J. Biochem.*, 1 (1967) 334.
6 H.R. Besch, Jr., J.C. Allen, G. Glick and A. Schwartz, *J. Pharmacol. Exptl. Therap.*, 171 (1970) 1.
7 T. Akera, F.S. Larsen and T.M. Brody, *J. Pharmacol. Exptl. Therap.*, 170 (1969) 1.
8 K. Nagai, F. Izumi and H. Yoshida, *J. Biochem.*, 59 (1966) 295.
9 H. Yoshida, K. Nagai, T. Ohashi and Y. Nakagawa, *Biochim. Biophys. Acta*, 171 (1969) 178.
10 R. Rodnight, D.A. Hems and B.E. Lavin, *Biochem. J.*, 101 (1966) 502.
11 K.S. Lee and D.H. Yu, *Biochem. Pharmacol.*, 12 (1963) 1253.
12 R.F. Palmer and B.R. Nechay, *J. Pharmacol. Exptl. Therap.*, 146 (1964) 92.
13 K. Repke, *1st Intern. Pharmacol. Meeting, Stockholm, 1961*, Vol. 3, Pergamon Press, Oxford, p.47.
14 S.L. Bonting, N.M. Hawkins and M.R. Canady, *Biochem. Pharmacol.*, 13 (1964) 13.
15 H.D. Brown, *Biochim. Biophys. Acta*, 120 (1966) 162.
16 R.L. Klein, *Biochim. Biophys. Acta*, 73 (1963) 488.