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EFFECTS OF PROSTAGLANDINS ON THE ATPase ACTIVITIES OF SYNAPTOSOMES

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Evidence now suggests that prostaglandins may be involved in regulating noradrenaline release at peripheral synapses (1). However, effects of prostaglandins on cholinergic transmission and on neurotransmitter release in the CNS have received less attention.

The release of acetylcholine by the myenteric plexus-longitudinal muscle preparation of the guinea-pig ileum (2) and by cerebral cortex slices (3) has been assumed to be mediated through inhibition of Na^+,K^+-activated, Mg^{++}-dependent ATPase (Na^+,K^+-ATPase). If this hypothesis can be extended to transmitter release generally then regulation of the release by prostaglandins could involve this mechanism.

We report here experiments to determine the effects of prostaglandins on the ATPase activities of nerve terminals (synaptosomes) isolated from the CNS.

Methods:

Synaptosomes were prepared from rat cerebral cortex, all procedures being performed at 0-4°C. The tissue was homogenised in 9 volumes 0.32 M sucrose containing 1 mM EDTA, using a glass homogeniser fitted with a teflon pestle of clearance 0.23 mm. The pestle was rotated at 840 r.p.m. The homogenate was centrifuged at 1000 g for 10 min, the supernatant was removed and the pellet washed with

0.32 M sucrose. The supernatant and washings were centrifuged at 10,000 g for 20 min and the pellet was washed with, and then resuspended in 0.32 M sucrose solution (3 ml/g fresh tissue) and layered on a discontinuous gradient consisting of 6 ml 1.2 M and 6 ml 0.8 M sucrose, and centrifuged at 53,500 g for 2 h. The synaptosome layer was removed, adjusted to a sucrose concentration of 0.4 M by the slow addition of 0.2 M sucrose, and centrifuged at 100,000 g for 30 min to obtain the synaptosome pellet. In some experiments the pellet was resuspended in 4.5 volumes 0.32 M sucrose and 4.5 volumes 0.32 M sucrose containing 0.4% lubrol and 2 mM EDTA added. The mixture was homogenised and allowed to stand on ice for 15 min before centrifuging it at 100,000 g for 30 min to obtain the lubrol-insoluble and soluble fractions as supernatant and pellet respectively.

For ATPase assays the synaptosomes were resuspended in 9 volumes 50 mM imidazole/HCl buffer pH 7.4 and 0.2 ml samples, each containing 0.1 - 0.2 mg protein, were added to buffered media. The media contained NaCl (150 mM), KCl (10 mM) and $MgCl_2$ (5 mM) for total ATPase, $MgCl_2$ (5 mM) for Mg^{++}-ATPase and $MgCl_2$ (5 mM) and NaCl (150 mM) for Na^+-ATPase. The mixtures, (final volume 0.9 ml) were pre-incubated for 15 min at 37°C before starting the reaction by adding 0.1 ml Tris ATP (4 mM final concentration). Sodium dodecyl sulphate (1 ml, 0.8%) was used to stop the reaction, usually after 10 min but a period of 30 sec was used for the kinetic studies. The phosphate contents of the clear solutions were determined by the method of Bonting, Simon and Hawkins (4). When used, drugs were added to the pre-incubation media. Na^+K^+-ATPase activity was calculated by subtracting the Mg^{++}-ATPase activity from the total activity, and the Na^+-ATPase activity was calculated by subtracting the Mg^{++}-ATPase activity from the activity determined in the presence of NaCl and $MgCl_2$.

Results and discussion

Table 1

Na^+K^+-ATPase Activities of Synaptosomes and Synaptosome-Extracts

Fraction	ATPase Activity (μmol Pi. mg Pr^{-1} h^{-1})			
	Na^+K^+	Na^+K^+, Ouab.	Na^+	Na^+, Ouab.
Synaptosome	$8.7\pm0.8_{(6)}$	$1.9\pm0.3_{(6)}$	$2.2\pm0.3_{(6)}$	$2.2\pm0.3_{(6)}$
Lubrol-Sol.	$28.3\pm9.6_{(5)}$	$9.5\pm0.1_{(3)}$	$8.3\pm0.9_{(5)}$	$8.9\pm0.9_{(3)}$
Lubrol-Insol.	$17.6\pm7.9_{(6)}$	$0.5\pm0.1_{(5)}$	$0.4\pm0.2_{(6)}$	$1.3\pm1.4_{(5)}$

The numbers of observations are given in parenthesis. The concentration of ouabain was 1 mM.

Table 1 shows the Na^+,K^+-ATPase activities of synaptosomes and fractions prepared from them. In contrast to results we have obtained using the same assay technique with erythrocyte gohsts prepared by the method of Wheeler and Whittam (5) ouabain did not inhibit the activity completely in the synaptosome fraction. The use of longer pre-incubation periods during which ouabain was in contact with the ATPase preparation, either in the presence or absence of K^+, did not increase the degree of inhibition significantly, and using another method (6) to assay the enzyme gave similar results. In none of the experiments did ouabain alter the Mg^{++}-

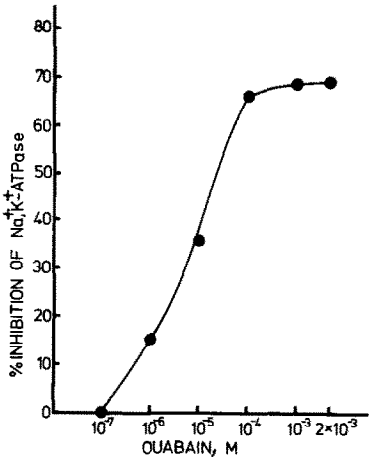

Fig. 1 : Effects of ouabain on Na^+K^+-ATPase. Na^+,K^+-ATPase activity was calculated by subtracting the Mg^{++}-ATPase activity from the total activity. Total ATPase activity was determined by incubating synaptosomes in imidazole/HCl buffer containing NaCl, KCl and $MgCl_2$ with or without ouabain, for 15 min before adding Tris-ATP.
Mg^{++}-ATPase activity was determined in the absence of NaCl and KCl.

ATPase activity. Fig. 1 shows that the concentration of ouabain was sufficiently high to inhibit the enzyme maximally.

In all three preparations the ouabain-insensitive activity was similar in magnitude to the degree of activation of ATPase (above the Mg^{++}-ATPase activity) by Na^+ in the presence of Mg^{++}. Furthermore this Na^+-stimulated activity was not significantly inhibited by ouabain (1×10^{-6}-1×10^{-3}M), suggesting that an ouabain-insensitive, Na^+-activated Mg^{++}-dependent ATP hydrolysing system (Na^+-ATPase for convenience) is present in the nerve terminals. An alternative explanation is that the endogenous K^+ which is present in the fractions (approx. 4 µM) is bound sufficiently firmly to the Na^+,K^+-activated enzyme to render it insensitive to ouabain. However, the concentration of endogenous K^+ in the lubrol-insoluble fraction was similar to that of the other preparations yet there was no activation of ATPase by Na^+ in that fraction. Replacement of Na^+ by choline, tris or sucrose did not activate the ATPase so that changes in ionic strength or osmolality are unlikely to account

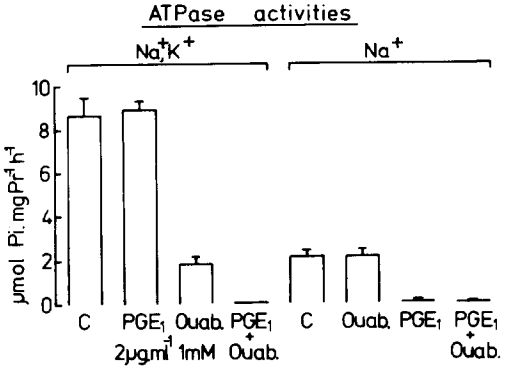

Fig. 2 : Effects of PGE_1 and ouabain on Na^+,K^+-ATPase and Na^+-ATPase activities of synaptosomes. The activities of the enzymes were determined as described in the text. Each result is the mean ± sem from 3 - 6 experiments.

for our results. Kinetic studies indicated that with respect to ATP, the Km for the Na^+-activated component (1.3×10^{-3}M) was significantly different from that of the Na^+,K^+-ATPase (4.0×10^{-4}M) but not significantly different from the Km for the residual Na^+,K^+-

ATPase activity determined when ouabain was present. This finding supports the concept of a Na^+-ATPase but does not preclude the possibilities firstly, that it could be an integral component of the Na^+,K^+-activated system or secondly that Na^+ influences the characteristics of the Mg^{++}-ATPase although, in view of the results with PGE_1 this seems unlikely.

Table 2

Effects of Prostaglandins on the ATPase activities of synaptosomes

Condition	ATPase Activity (μmol Pi. mg Pr^{-1} h^{-1})		
	Na^+K^+	Na^+	Mg^{++}
Control	$8.7\pm0.8_{(6)}$	$2.2\pm0.3_{(6)}$	$6.0\pm0.5_{(6)}$
PGE_1 (2 μg. ml^{-1})	$8.9\pm0.4_{(3)}$	$0.0\pm0.0_{(3)}$*	$6.2\pm0.2_{(4)}$
PGE_2 (1 μg. ml^{-1})	$6.2\pm1.7_{(3)}$	$2.6\pm0.3_{(3)}$	$5.8\pm0.2_{(3)}$
$PGF_2\alpha$ (0.6 μg. ml^{-1})	$6.8\pm2.0_{(3)}$	$1.6\pm0.5_{(3)}$	$6.4\pm0.2_{(3)}$

* $P < 0.001$. The numbers of observations are given in parenthesis.

PGE_1 (2 μg/ml) did not alter synaptosome Na^+,K^+-ATPase activity (Table 2). However, PGE_1 and ouabain together completely abolished it (Fig 2). If the ouabain-insensitive Na^+,K^+-ATPase activity is indeed due to the Na^+-activated component then PGE_1 should prevent the activity detected when Na^+ and Mg^{++} are added (without K^+) and this was the case. A dose-response curve showed that inhibition of Na^+-ATPase was maximal at 2 μg PGE_1/ml. Fig. 2 also shows that the Na^+-ATPase was not significantly inhibited by ouabain. In all experiments neither PGE_1 or ouabain altered Mg^{++}-ATPase activity detectably. Prostaglandins E_2 and $F_2\alpha$ (1.0 and 0.6 μg/ml respectively) did not significantly alter the activities of the enzymes in three experiments (Table 2) and the same result was obtained in one experiment performed using higher concentrations (4.0 and 2.4 μg/ml respectively).

Since PGE_1 did not inhibit Na^+,K^+-ATPase in spite of its effect

on the Na^+-activated component our results can be explained on the basis of the Na^+-dependent phosphorylation/phosphorylated enzyme/ K^+-dependent dephosphorylation model of Na^+,K^+-ATPase. We would assume that the formation of the phosphorylated intermediate in the presence of Na^+, Mg^{++} and ATP can result subsequently in the release of phosphate in synaptosome preparations even in the absence of exogenous K^+. PGE_1 then prevents this release (stabilises the phosphorylated intermediate) whereas K^+ not only causes the complete dephosphorylation normally, but also causes it to occur in the presence of PGE_1. Ouabain acts, as has been suggested, (7) at the K^+ site, preventing the K^+-induced component of the dephosphorylation but not the Na^+ activated phosphate release or the inhibition of that release by PGE_1.

Whatever the mechanism responsible for the effects we have observed, if ATPases of the nerve terminal are indeed involved in regulating neurotransmitter release then the effect of PGE_1 reported here takes on added significance.

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