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Control of adenylate kinase by prostaglandins E_2 and E_3

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ADENYLATE kinase (myokinase) catalyses the reversible reaction ATP + AMP $\rightleftharpoons$ 2 ADP. In skeletal muscle it is considered to function in providing ATP when ATP resources are exhausted during a strenuous contraction. It is assigned a similar function in cells, like blood platelets, that are devoid of creatine kinase. It is likely that ADP formation is the reactions role under normal conditions.[1]

In this communication evidence is presented to show that prostaglandins E_2 and E_3 control the adenylate kinase reaction in muscle, platelets and brain. Prostaglandin E_2 was found to stimulate the enzyme, increase its affinity for ATP and AMP and, furthermore, to increase the maximum velocity of the reaction. Prostaglandin E_3 caused the enzyme to be inhibited by ADP. The higher the dose pGE_3, the smaller the concentration of ADP that inhibits.

Methods and results

Pure muscle adenylate kinase (Sigma)* was assayed in the direction 2 ADP $\rightarrow$ ATP + AMP by measuring the increases in absorbance at 340 nm in a reaction mixture containing 0·5 units enzyme/ml, 50 mM tris–HCl (pH 7·5), 5 mM $MgSO_4$, 10 mM glucose, 10 units hexokinase, 10 units glucose-6-phosphate dehydrogenase and 0·2 mM NADP.[1,2] The reaction was started by adding various concentrations of ADP ranging from 0·05 mM to 2 mM. The action of prostaglandin E_2 and E_3 on the enzyme activity is shown in Fig. 1. It shows the substrate inhibition with pGE_3 and increased V_{max} in presence of pGE_2.

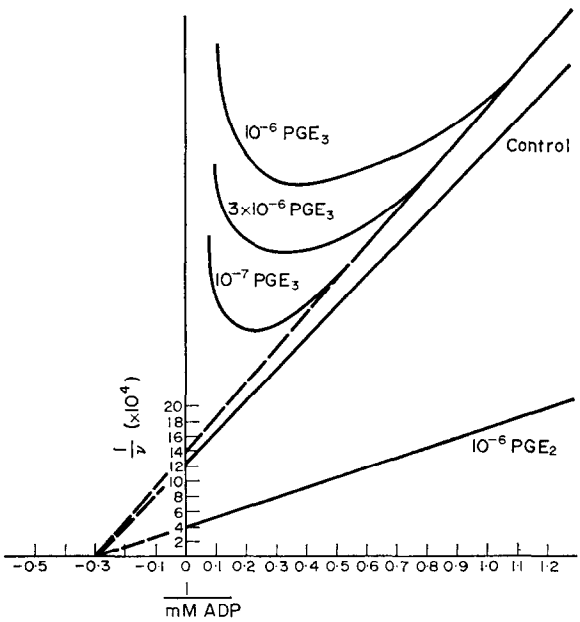
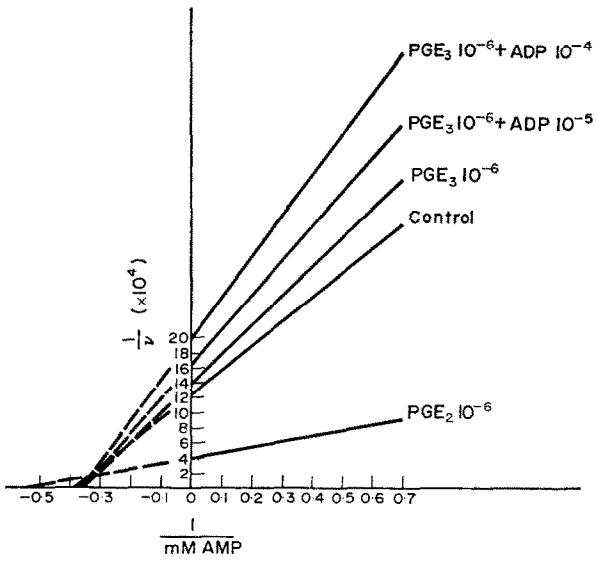


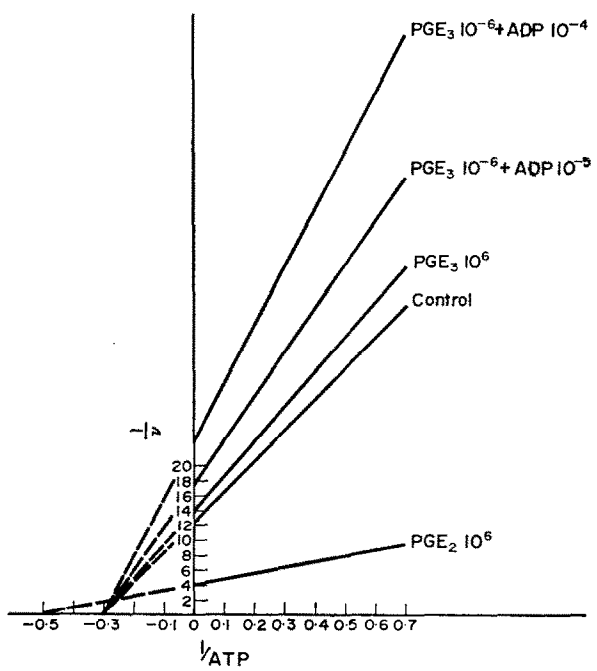
FIG. 1. Action of pGE_2 and pGE_3 on pure muscle adenylate kinase assayed in the direction ADP $\rightarrow$ ATP + AMP.

Measurement of the reverse reaction ATP + AMP $\rightarrow$ 2 ADP was achieved by assaying [^{14}C]-labelled ADP formed from labelled AMP + non-radioactive ATP. The reaction mixture (0·1 ml) contained 2 mM 8-[^{14}C]AMP (50,000 counts/min), 2 mM ATP, 50 mM tris buffer (pH 7·5), 5 mM $MgSO_4$ and 2 units/ml of pure adenylate kinase. After incubation at 37° for 15 min the reaction was stopped by transferring the tubes into ice and adding 0·01 ml of cold 3 N trichloracetic acid followed

* Sigma contains 5 units adenosine, 5′ diphosphatase/mg protein.



(a)



(b)

FIG. 2. Action of pGE_2 and pGE_3 on pure muscle adenylate kinase assayed in the direction ATP + AMP → ADP.

(a) shows the effect of varying the concentration of AMP in presence of excess ATP.

(b) shows the effect of varying the concentration of ATP in presence of excess AMP.

by 0·01 ml of 3 M tris buffer. The reaction mixture was resolved on cellulose thin-layer chromatoplates developed in butanol–acetone–acetic acid–water–ammonia (5%) (35:25:15:15:10, by vol.).[4] Areas in the chromatogram corresponding to carrier ADP were scraped for measuring radioactivity by liquid scintillation counting with a Intertechnique SL.40 liquid scintillation spectrometer. To determine substrate affinities the concentration of one nucleotide was varied (0·05–0·5 mM) while the others were in excess. Figure 2 shows that pGE_2 increases V_{max} and decreases K_m for AMP and ATP. It also shows that inhibition by ADP, conferred by pGE_3, is purely competitive.

The same method was used to measure the enzyme activity in whole brain homogenates and in blood platelet suspensions. Adenylate kinase is the only enzyme that converts labelled AMP to ADP. K_m and V_{max} values computed from Lineweaver–Burk plots are shown in Table 1.

TABLE 1

Enzyme source	Addition	$K_m \times 10^{-4}$M ATP	AMP	ADP	V_{max}*
Purified muscle enzyme	None	3·3	2·6	3·3	800
	pGE_2 10^{-6}M	2·0	1·8	3·3	2400
	pGE_3 10^{-6}M	3·4	2·8	3·4	720
Human platelet† suspension	None	3·2	3·0	—	4·5
	pGE_2 10^{-6}M	2·9	2·3	—	9·1
	pGE_3 10^{-6}M	4·0	3·1	—	4·1
Rat brain homogenate	None	5·1	4·5	—	3·1
	pGE_2 10^{-6}M	3·6	3·6	—	5·9
	pGE_3 10^{-6}M	5·1	4·6	—	3·0

* V_{max} is expressed as μmoles ATP formed per minute per mg protein of the pure muscle enzyme. V_{max} values for platelets and rat brain represent μmoles of ADP form from ATP + AMP per minute per mg protein.

† Washed platelets were prepared as described in Ref. 3. They were frozen and thawed once to render the cell membranes permeable to nucleotides.

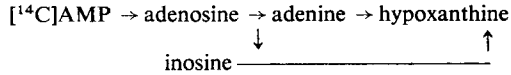
TABLE 2

Labelled nucleotide	Additions*	Percentage radioactivity ATP	ADP	AMP	Inosine + adenosine	Hypoxanthine
AMP	None	2	5	15	10	68
	apyrase					>94%
	pGE_2	24	38	30	6	2
	pGE_2 + apyrase	6	4	76	8	6
	pGE_3	15	15	38	10	22
	pGE_2 + pGE_3	6	5	20	10	59
ATP	None	3·7	2·4	3·6	3·3	87
	pGE_2	40	38	17	3	2
	pGE_3	5	3	3	4	85
	pGE_2 + pGE_3	4	3·5	3	3	86

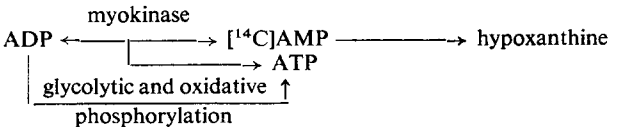
* Prostaglandins were added at a concentration of 5×10^6 M. Potato apyrase was used at 5 units of enzyme per ml of the reaction medium.

The following experiment shows the effects of prostaglandin E_2 on intracellular nucleotide pools. Washed platelets (5×10^7), frozen and thawed once, were incubated for 7 hr at 37° in 0·3 ml of a mixture containing 2 mM AMP (8-[^{14}C]50,000 counts/min), 2 mM ATP, 120 mM NaCl, 10 mM KCl, 10 mM tris–HCl (pH 7·4), 10 mM glucose, 3 mM $HgCl_2$ and 1 mM $CaCl_2$. Nucleotides, nucleosides and purine bases were resolved by cellulose thin-layer chromatography as described above. Permanent records of the chromatograms were made by autoradiography. The effects of various additions on the distribution of radioactivity are shown in Table 2. In the simple reaction medium most of radioactivity was in hypoxanthine.

Potato apyrase (ADP → AMP) more completely converted nucleotides to hypoxanthine.



Addition of pGE_2 led to the retention of most radioactivity in AMP, ADP and ATP (30, 38 and 24 per cent, respectively). It was presumed that adenylate kinase was stimulated by pGE_2 to such a degree as to keep the radioactivity shuttling between the three nucleotides.



In the presence of both pGE_2 and potato apyrase,* 76 per cent of radioactivity remained in AMP; only 14 per cent was incorporated into inosine and hypoxanthine. pGE_2 action seemed to cause added AMP to be channelled by adenylate kinase to ADP, which in turn was hydrolysed by apyrase back to AMP. The affinity of the stimulated enzyme for AMP and the increased turnover could be of such magnitude as to channel the mononucleotides along the circular pathway away from degradation to hypoxanthine.

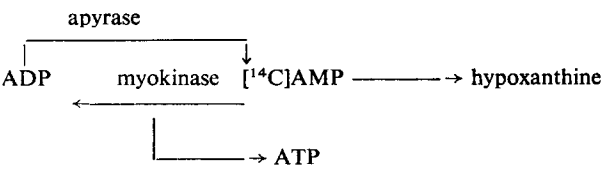


Table 2 shows that pGE_2 also promotes the maintenance of a large total nucleotide pool and, furthermore, expands the ADP fraction if the source of radioactivity is ATP rather than AMP.

pGE_3 in low concentration has little effect apart from reversing the action of pGE_2.

Discussion

A sensitive system for controlling adenylate kinase is provided by pGE_3; it gradually switches off enzyme activity as more ADP is formed. Under the influence of pGE_3 the reaction is sensitive to the size of the ADP pool that is accessible to the enzyme. When the pool is small, the reaction proceeds uninhibited. As the pool enlarges ADP formation (from AMP + ATP) gets slower until, at a certain ADP concentration, the reaction completely stops. It is thus possible that control of adenylate kinase by pGE_3 functions more (only?) when the enzyme is stimulated by pGE_2.

Prostaglandin E_2 enhances platelet aggregation.[5] This could be due to increased ADP formation by the stimulated adenylate kinase. The proposed site of action of prostaglans E_2 and E_3 could be elucidated by analogy with their action on smooth muscle, for there is evidence that the relative concentrations of adenine nucleotides may affect contractility. In studying the relaxing effect of adrenalin on taenia coli, Bueding *et al.*[6] found that, coinciding with hyperpolarisation and inhibition of contraction, adrenalin increases the tissue concentration of ATP and creatine phosphate. Potassium induced contracture of taenia coli is relaxed by ATP and AMP.[7] Low concentration of ATP causes relaxation of the virgin cat uterus and excitation in the early pregnant cat uterus.[8] ATP may be the transmitter of sensory nerve antidromic vasodilatation (mediating axon reflex).[9] ADP depresses the spontaneous activity of frog stomach annular muscle.[10]

From skeletal muscle research we know that Ca^{2+} control of contraction functions only when the concentration of Mg ATP is more than 10^{-5} M.[11] Mg ATP in addition to being a substrate for

* From rabbit muscle 800 units/mg protein batch No. M-3003.

myosine ATPase, might possibly attach itself to another site of lower affinity, inhibiting the ATPase.[12,13] At low concentration of ATP, contraction may proceed without release of Ca^{2+}. The function and turnover of the actin bound ADP is not yet clear, but it is well known that ADP is a competitive inhibitor of pure myosin ATPase.[14]

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Folic acid and convulsions in the rat

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MEGALOBLASTIC anaemia may occur in patients undergoing drug treatment for major epilepsy.[1] This anaemia responds to folic acid, but not to Vitamin B_{12}.[2] In fact the majority of drugs which are effective in preventing convulsions in man have been reported to produce a folic acid deficiency anaemia. It is therefore possible that folic acid plays a role in the function of the nervous system and affects its susceptibility to epilepsy. In support of this is the observation that children treated with anti-epileptic drugs may suffer progressive mental deterioration, which is accompanied by low serum folate levels. Mental function may improve in these patients when folic acid is administered[3] but there is evidence that this may aggravate the epilepsy.[4] However, this deleterious effect of folic acid in epilepsy has recently been disputed.[5] The following is an experiment in which an antimetabolite, methotrexate, is used to decrease utilisation of folic acid in the tissues. The effect of the drug is to inhibit folate reduction,[6] so that dihydrofolate and tetrahydrofolate production is inhibited, but folate absorption from the intestine is primarily unimpaired. The susceptibility to chemically induced seizures was estimated by measuring the time of onset and severity of fits following administration of 50 mg/kg of leptazol. Adult white Wistar rats of either sex, weighing 105–155 g were used. Methotrexate 4 mg/kg was injected intraperitoneally into test animals and isotonic saline was injected into controls. Two further groups of controls were used: (i) 20 mg/kg folic acid was injected with 4 mg/kg