A CALCIUM SUPPORTED ADENYLYL CYCLASE ACTIVITY IN THE PARS DISTALIS OF THE DOGFISH PITUITARY

D.J. Deery
Department of Anatomy, University of London
King's College, Strand, London WC2R 2LS

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SUMMARY

A magnesium-dependent adenylyl cyclase activity was found in each of the four lobes of the dogfish pituitary. An unusual feature of the enzyme system of the three lobes of the pars distalis was a marked enhancement of fluoride-activation in the presence of calcium ions at concentrations usually found to inhibit adenylyl cyclase systems. With the neurointermediate lobe enzyme the fluoride-activation was inhibited by calcium ions. In the absence of magnesium ions the enzyme activity of the pars distalis was supported by calcium ions, the calcium-supported activity being greater than the magnesium-supported activity.

INTRODUCTION

Adenylyl cyclase systems have an absolute requirement for a divalent cation and both Mg^{++} and Mn^{++} support enzyme activity in the majority of tissues (1,2). Although Ca^{++} is required in trace amounts for adenylyl cyclase activity of porcine renal medullary membranes (3) and for hormonal stimulation in some tissues (4-6), at higher concentrations Ca^{++} has been found to be inhibitory.

A hormone-sensitive adenylyl cyclase activity has been found in the pituitary of the dogfish, Scyliorhinus canicula, and has been studied in this laboratory (7). The pituitary of this elasmobranch is subdivided into four distinct lobes. The pars distalis is composed of rostral, median and ventral lobes and the pars intermedia and pars nervosa combine to form the neurointermediate lobe (8).

This report describes the ionic requirements of the adenylyl cyclase of each lobe of the dogfish pituitary and provides evidence to suggest that Ca^{++} ions may be more closely involved than Mg^{++} ions in the maintenance of adenylyl cyclase activity in each of the lobes of the pars distalis of this gland. Also, evidence is presented to suggest that this may not be true for the enzymes of the pars distalis of the rat and of a teleost fish, Carassius auratus.

MATERIALS AND METHODS

The $[\alpha\text{-}^{32}P]$ ATP, of specific activity 500-3000 Mci/mmol, was obtained from the Radiochemical Centre, Amersham, Bucks. Crystallised bovine plasma albumin was obtained from BDH Biochemicals Ltd., Poole, Dorset. All other enzymes and biochemicals were supplied by Sigma Chemical Co., (London) Ltd., Kingston-upon-Thames, Surrey.

Mature female dogfish (Scyliorhinus canicula L.) were obtained by trawling off the Devon coast. The animals were killed by decapitation and the brain was removed and immersed in dogfish ringer (pH 7.4) at 4°C. Each lobe of the pituitary was separately dissected and lobes were collected in separate vessels containing cold dogfish ringer. The composition of the ringer was as follows:- 2 mM NaCl, 3.3 mM KCl, 1.8 mM $CaCl_2$, 2.2 mM $MgCl_2$, 3.5 mM Na_2SO_4, 5.9 mM $NaHCO_3$, 50 mM D-glucose and 450 mM urea. The ringer was gassed with O_2:CO_2 (95:5) for ten minutes before use.

The pituitary lobes were collected, in groups of 20, and homogenised in 2.0 ml of 25 mM Tris (pH 7.6) containing 1 mM EDTA, 5 mM $MgCl_2$ and 10% dimethyl sulphoxide. The homogenate was centrifuged for ten minutes, in a chilled centrifuge bowl, at top speed in a bench centrifuge. The precipitate was washed twice with 2.0 ml of the above medium. Aliquots of 0.2 ml were then added to small glass vials which were stoppered, the contents snap frozen in an acetone/dry ice mixture, and stored at -70°C until required for the adenylyl cyclase assay.

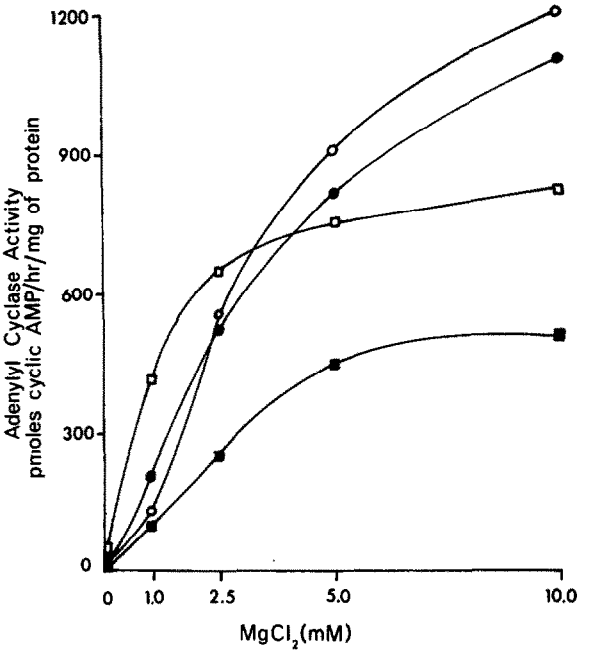
The reaction was initiated by the addition of 5-15 μg of the pituitary lobe homogenate protein to the reaction mixture. The composition of the reaction mixture was as follows: 1.10^{-5} M $[\alpha\text{-}^{32}P]$ ATP, 25 mM Tris (pH 7.6), 1 mM EDTA, 5 mM $MgCl_2$, 3 mM theophylline, 0.1% bovine plasma albumin and an ATP regenerating system consisting of 25 mM phosphocreatine and 1 mg/ml creatine phosphokinase in a final volume of 0.1 ml. This is essentially the method described by Krishna et al. (9) as modified by Pohl et al. (10), with the exception that the substrate concentration was lowered to 1.10^{-5} M. The reaction tubes were incubated at 30°C for 40 minutes and the reaction was stopped by boiling for 4 minutes after which 3 mM cyclic AMP was added to each tube as carrier. The $[^{32}P]$ cyclic AMP formed was separated on neutral alumina columns (5 cm x 1 cm) as described by Ramachandran (11) and was eluted from the columns in 10 mM Tris (pH 7.4) in a volume of 6.0 ml. The radioactivity from each column was determined directly in a liquid scintillation spectrometer Model 3330, Packard Instrument Co. Inc., 2200 Warrenville Road, Downers Grove, Ill. 60515, U.S.A.

The protein content of the pituitary lobe homogenates was determined by the procedure of Lowry et al. (12) using crystallised bovine plasma albumin as standards.

RESULTS AND DISCUSSION

Cyclic AMP accumulation for this system was linear up to forty minutes of incubation time and was proportional to the amount of pituitary lobe protein present (7). The results in Fig. 1 show that the fluoride-stimulated enzyme activity of each pituitary lobe was Mg^{++}-dependent, a rapid rise in activity occurring up to 10 mM of Mg^{++} ions. The basal or unstimulated activity showed a similar dependence except that the increase continued up to 10 mM of Mg^{++} ions. This is in good agreement with the Mg^{++}-dependence reported for mammalian systems (13).

FIG.1

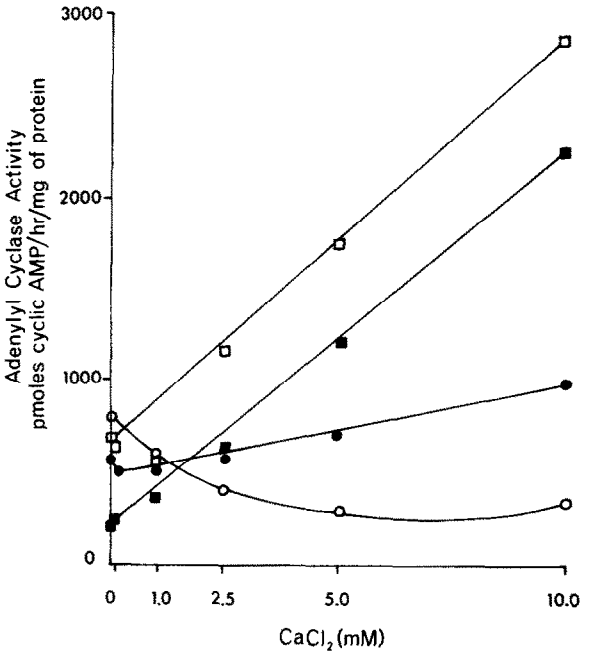


Effect of varying concentrations of $MgCl_2$ on the sodium fluoride (10 mM) stimulated adenylyl cyclase activity of the median (●), rostral (□), ventral (■) and neurointermediate lobes (○) of the dogfish pituitary. EDTA was omitted from the reaction mixture and each value is the mean of triplicate observations.

When Ca^{++} ions were present, in the concentration range 1-10 mM, the Mg^{++}-supported fluoride activation was increased for each of the three adenylyl cyclase preparations of the pars distalis (Fig. 2). This is in contrast to the usual effect of Ca^{++} on adenylyl cyclase systems (14), and to the effect of Ca^{++} on the enzyme system of the neurointermediate lobe (Fig. 2). That this effect of Ca^{++} is unique to the pars distalis of the dogfish pituitary is shown by Fig. 3. This demonstrates that Ca^{++} was inhibitory to the adenylyl cyclase of the pars distalis of the pituitary of the rat and of the teleost fish, Carassius auratus.

The results in Fig. 4 demonstrate that Ca^{++} was capable of supporting adenylyl cyclase activity of the dogfish pars distalis

FIG. 2



Effect of varying concentrations of $CaCl_2$ on the sodium fluoride (10 mM) stimulated adenylyl cyclase activity of the median (●), rostral (□), ventral (■) and neurointermediate lobes (o) of the dogfish pituitary. EDTA was omitted from the reaction mixture and each value is the mean of triplicate observations.

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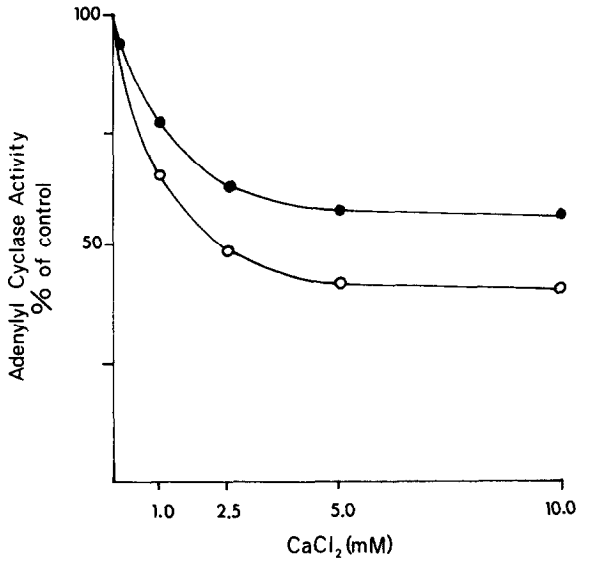
even in the absence of Mg^{++} ions and that the Ca^{++}-supported activity was greater than the Mg^{++}-supported activity.

It was found by Birnbaumer et al. (15) in rat fat cells and Drummond and Duncan (16) in rat cardiac tissue that the substrate for the enzyme is MgATP and that free ATP is inhibitory to the reaction. It has also been suggested that there is a second, allosteric site for Mg^{++} and that Ca^{++} inhibits the enzyme by competing with Mg^{++} for this allosteric site. The increase in activity of the dogfish pars distalis enzyme in response to increasing Ca^{++} ion concentration in the presence of 5 mM Mg^{++} ion, at which concentration the enzyme is approaching saturation with regard to Mg^{++}, suggests that at least in this tissue Ca^{++} is not exerting its effect by competing with Mg^{++} for occupancy

FIG.3



Effect of varying concentrations of $CaCl_2$ on the sodium fluoride (10 mM) stimulated adenylyl cyclase activity of the pars distalis of the rat (●—●) and the goldfish (o—o) pituitary. EDTA was omitted from the reaction mixture and each value is the mean of triplicate observations.

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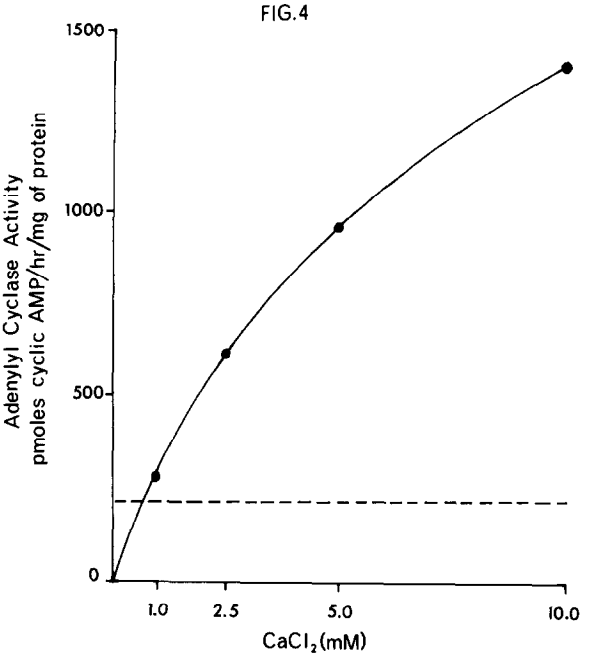
of an allosteric site. Instead it may be that a distinct allosteric site exists for Ca^{++} which would be able to functionally replace the Mg^{++} specific allosteric site, in the absence of Mg^{++}, so as to allow expression of enzyme activity (see Fig. 5). An alternative explanation might be that Ca^{++} by binding preferentially or more effectively to ATP counteracts more efficiently the inhibitory effect of free ATP. In this situation it has been suggested that F^- ion and hormones may act to lower the affinity of the enzyme for the inhibitory free nucleotide (14).

It therefore appears that the dogfish pars distalis adenylyl cyclases are unique among the vertebrate systems so far studied in respect to their ionic requirements. However, due to the lack of knowledge of pituitary regulation in the elasmobranch fishes it is unclear at present if these effects of Ca^{++} ions have any physiological significance.

FIG.4



Effect of varying concentrations of $CaCl_2$, in the absence of Mg^{++} ions, on the sodium fluoride (5 mM) stimulated adenylyl cyclase activity of the rostral lobe of the dogfish pituitary. The activation due to sodium fluoride (5 mM) in the present of Mg^{++} ions (5 mM) is also shown (- - -). In this experiment the particulate preparation was prepared in Mg^{++}-free Tris buffer. $MgCl_2$ was also omitted from the reaction mixture and the EDTA concentration reduced to 0.5 mM. Each value is the mean of triplicate observations.

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