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Activities and Some Properties of Adenylate Cyclase and Phosphodiesterase in Muscle, Liver and Nervous Tissues from Vertebrates and Invertebrates in Relation to the Control of the Concentration of Adenosine 3′:5′-Cyclic Monophosphate

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1. The basal and fluoride-stimulated activities of adenylate cyclase, and the maximal activities of 3′:5′-cyclic AMP phosphodiesterase and 3′:5′-cyclic GMP phosphodiesterase, together with the K_m values for their respective substrates, were measured in muscle, liver and nervous tissues from a large range of animals to provide information on the mechanism of control of cyclic AMP concentrations in these tissues. High activities of adenylate cyclase and cyclic AMP diesterase are found in nervous tissues and in the more aerobic muscles (e.g. insect flight muscles, cardiac muscle and some vertebrate skeletal muscles). The activities of these enzymes in liver are similar to those in the heart of the same animal. The K_m values for the enzymes from different tissues and animals are remarkably similar. 2. The comparison of cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase activities suggests that in vertebrate tissues only one enzyme (the high-K_m enzyme), which possesses dual specificity, exists, whereas in invertebrate tissues there are at least two phosphodiesterases with separate specificities. 3. A simple quantitative model to explain the control of the steady-state concentrations of cyclic AMP is proposed. The maximum increase in cyclic AMP concentration predicted by comparison of basal with fluoride-stimulated activities of adenylate cyclase is compared with the maximum increases in concentration produced in the intact tissue by hormonal stimulation: reasonable agreement is obtained. The model is also used to predict the actual concentrations and the rates of turnover of cyclic AMP in different tissues and, where possible, these values are compared with reported values. Reasonable agreement is found between predicted and reported values. The possible physiological significances of different rates of turnover of cyclic AMP and the different ratios of high- and low-K_m phosphodiesterases in different tissues are discussed.

The importance of cyclic AMP in the control of many physiological processes in diverse organisms from bacteria to man is now well established. The intracellular concentration of cyclic AMP is maintained at a steady-state value by its production from ATP by adenylate cyclase (EC 4.6.1.1) and conversion into AMP by cyclic AMP phosphodiesterase (EC 3.1.4.17). Changes in the concentration of cyclic AMP produced by physiological stimuli (e.g. hormones, nervous action) are achieved mainly through modifications in the activity of adenylate cyclase, whereas changes produced pharmacologically involve modifications (usually inhibition) of either cyclase or phosphodiesterase activities. Despite the obvious importance of these enzymes in the control of cyclic AMP concentrations in tissues throughout the Animal Kingdom, a systematic comparative study of their activities and properties has not been reported. This paper presents the results of such an investigation. In addition, a simple model to explain the control of the steady-state concentrations of cyclic AMP is described and the model is used to analyse the data reported in this and other work.

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Materials and Methods

Chemicals and enzymes

All chemicals and enzymes were obtained from Boehringer Corp. (London) Ltd., London W5 2TZ, U.K., except for the following: cyclic AMP, cyclic GMP, 5′-nucleotidase (EC 3.1.3.5) (freeze-dried powder from *Crotalus adamanteus* venom) and bovine serum albumin (fraction V) were obtained from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey KT2 7BH, U.K.; radiochemicals were obtained from The Radiochemical Centre,

Amersham, Bucks. HP7 9LL, U.K.; Triton X-100, dithiothreitol, propan-2-ol and ethyl acetate were obtained from BDH Chemicals, Poole, Dorset BH12 4NN, U.K.; EDTA and all inorganic chemicals were obtained from Fisons Scientific Apparatus, Loughborough, Leics. LE11 0RG, U.K. The anion-exchange resin, AG 1 (X8; 200–400 mesh, Cl^- form) was obtained from Bio-Rad Laboratories, Bromley, Kent BR4 1T3, U.K. Silica-coated plastic sheets (Polygram SIL N-HR/UV_{254}) were obtained from Camlab Ltd., Cambridge CB4 1TH, U.K.

Sources of animals

Mature animals were obtained from sources that have been described previously (Newsholme & Taylor, 1969; Sugden & Newsholme, 1973), with the exception or addition of the following: rosechafers and honey-bees were bred at the Department of Zoology, Oxford; trout were obtained from the Trout Hatchery (Bibury, Glos., U.K.). Apart from the rats, which were male, and the bees, which were workers, male and female animals were used indiscriminately. All the animals had access to food and water for at least 24h before death.

Preparation of homogenates

The animals were killed and tissues removed as soon as possible after death. Scallops, lobsters, bees and blowflies were cooled on ice before they were killed. The tissues were homogenized in ground-glass homogenizers with 10–50vol. of extraction medium at 0°C. Some vertebrate muscles were first homogenized in a Silverson homogenizer (Silverson Machines Ltd., Chesham, Bucks. HP5 1PQ, U.K.) before the ground-glass homogenizer. The extraction medium consisted of 40mM-Tris/HCl, 8.7mM-$MgCl_2$, 1mM-dithiothreitol and 1mg of bovine serum albumin/ml at pH7.5. In addition, 0.1% (v/v) Triton X-100 was included in the extraction medium for assays of phosphodiesterase from nervous tissues and, in some cases, adenylate cyclase from nervous tissues (see the Results section).

For the assay of adenylate cyclase activities, the tissues were extracted immediately after dissection. For the assay of phosphodiesterase activities, tissues were stored at 0°C for up to 2h before extraction. Preliminary experiments established that for a number of tissues from different animals there was no change in phosphodiesterase activity if extraction was delayed for 3h. Even after the tissue was stored for 24h (at 0°C), there was no marked change in activity. Enzyme-activity assays were always performed within 15min of the preparation of the homogenate.

For the measurement of fluoride-stimulated adenylate cyclase activity, tissues were initially homogenized in the absence of NaF as described above. One portion of the homogenate was diluted with the same extraction medium and another with a similar medium, except that it contained NaF and supplementary $MgCl_2$, so that the final concentrations were 12mM-NaF and 12.3mM-$MgCl_2$. This procedure enabled the fluoride-stimulated activity to be compared with the basal activity for the same extract. Since the effect of fluoride develops over a period of time (Perkins & Moore, 1971), the diluted homogenates were incubated at 30°C for 10min before enzyme-activity assay. Preliminary experiments with homogenates from mouse, rat and locust tissues showed that at this time the fluoride-stimulated activity of adenylate cyclase was maximal. Further incubation of the homogenates often resulted in a fall in both basal and fluoride-stimulated activities.

Assay of enzyme activities

Adenylate cyclase. The assay for adenylate cyclase was rapid and required very small quantities of tissue. It was developed from that described by House *et al.* (1972). The conversion of [^{3}H]ATP into cyclic [^{3}H]AMP was measured in the presence of an ATP-regenerating system and unlabelled cyclic AMP. The incubation medium consisted of 32mM-Tris/HCl, 7.6mM-$MgCl_2$, 1.8mM-dithiothreitol, 0.6mM-EDTA, 2.5mM-cyclic AMP, 16mM-creatine phosphate, 0.8mg of creatine kinase (EC 2.7.3.2)/ml and 0.3mg of bovine serum albumin/ml at pH7.5. At least five different equimolar concentrations (0.1–2mM) of $MgCl_2$ plus [2-^{3}H]ATP (1.6μCi) were used to obtain values of V and K_m for the enzyme. The fluoride-stimulated activity of adenylate cyclase was measured by using the incubation medium described above, except that it contained 10mM-NaF, 9.2mM-$MgCl_2$ and 0.5mM-[2-^{3}H]ATP. The reaction was started by addition of a volume (6μl) of homogenate to the incubation medium in a small glass tube so that the total volume was 20μl. The mixture was incubated for 5–20min before addition of 3μl of 2M-$HClO_4$ containing adenine, adenosine, hypoxanthine, inosine, ATP, ADP and AMP at approx. 5mM concentrations. Shorter incubation times were necessary when the concentration of the substrate was low, or the activities in the homogenate of ATPases,* 5′-nucleotidase and AMP deaminase were high. Control tubes were usually incubated for the same period of time, but $HClO_4$ was added before the tissue homogenate. The tubes were centrifuged at 2000g for 5min and the supernatant was used for subsequent chromatography. Preliminary experiments established that the rate of accumulation of cyclic [^{3}H]AMP was linear during the period of the assay. There was no accumulation of cyclic [^{3}H]AMP during the incubation period in the control tubes.

* Abbreviations: ATPase, adenosine triphosphatase; EGTA, ethanedioxybis(ethylamine)tetra-acetate.

Some batches of [^{3}H]ATP were contaminated with significant amounts of cyclic AMP. This was decreased by preincubation of the [^{3}H]ATP with a commercial phosphodiesterase preparation. Suitable conditions were as follows. Commercial phosphodiesterase (2μg) was incubated at 30°C with 100μl of the above assay medium (from which cyclic AMP was omitted). After 30–45min, unlabelled cyclic AMP was added so that its final concentration was 3.5mM. Samples of this medium were used as the assay medium for the adenylate cyclase assay. The assay was started at once. (The activity of phosphodiesterase added during the preincubation was no greater than that added to the assay owing to the presence of phosphodiesterase in the extract.)

Cyclic AMP was separated from ATP and degradation products of ATP by t.l.c. on silica-coated plastic sheets (10cm × 20cm), which were impregnated with a fluor absorbing at 254nm. A portion (10μl) of the $HClO_4$ supernatant was applied in four or five stages to a line of length 11mm which was 13mm from the base of the chromatogram. The chromatogram was developed with propan-2-ol/ethyl acetate/8M-NH_3 (9:4:3, by vol.) (Woods & Waitzman, 1970). To obtain compact spots and satisfactory separation, an initial development of 5–10min was used. The chromatogram was then dried in a stream of cold air before the main development (1–1½h) was carried out. The chromatogram was first dried in air at room temperature (19–21°C) and then at 60°C in an oven. It was cooled in a desiccator. The cyclic AMP spot, which was located under u.v. light, was cut out and placed face-up in a liquid-scintillation vial containing scintillation fluid [20mg of 2,5-diphenyloxazole and 0.5mg of 1,4-bis-(5-phenyloxazol-2-yl)benzene in 5ml of toluene]. The radioactivity was measured in a liquid-scintillation counter. Typical R_F values were as follows: purine nucleotides (except cyclic nucleotides), 0–0.03; inosine, 0.24; cyclic AMP, 0.35; hypoxanthine, 0.43; adenosine, 0.56; adenine, 0.64. Control tubes were treated in a similar manner and the radioactivity in the purine nucleotide (origin) spot provided a measure of the radioactivity in the ATP at zero time, so that the proportion of ATP converted into cyclic AMP could be calculated.

Phosphodiesterases. The activities of cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase were assayed by a radiochemical procedure in which the substrate and products were separated by using an anion-exchange resin. Since 5′-nucleotidase was included in the assay system, the products of the reaction were adenosine and guanosine rather than the nucleotides. The incubation medium consisted of 50mM-Tris/HCl, 6mM-$MgCl_2$, 2.5mM-dithiothreitol, 0.05mg of 5′-nucleotidase/ml, 0.23mg of bovine serum albumin/ml at pH7.5. At least 12 different concentrations of cyclic [8-^{3}H]AMP or cyclic [8-^{3}H]GMP (0.15–500μM) were included in each assay. The reaction was started by adding a volume (30μl) of homogenate to the incubation medium in a small polystyrene tube, so that the total volume was 130μl. Tissue homogenate was omitted from control tubes. (Such controls measured any phosphodiesterase activity in the 5′-nucleotidase preparation.) Controls were carried out for most of the substrate concentrations used.

The tubes were incubated for 10–15min. Longer times (up to 60min) were used if the activity of the enzyme was low. The assay was stopped by the addition of 1.2ml of a slurry of anion-exchange resin. [The slurry was made up with resin and water, 1:1 (v/v), although if the water content of the 'settled' resin is taken into account the ratio is 1:2.] The tubes were centrifuged briefly (2000g for 5min) and a portion (0.5ml) of the supernatant was added to 10ml of scintillation fluid [which contained 40mg of 2,5-diphenyloxazole, 1mg of 1,4-bis-(5-phenyloxazol-2-yl)benzene, 6.25ml of toluene and 3.75ml of 2-ethoxyethanol]. Radioactivity was measured in a liquid-scintillation counter.

Since the anion-exchange resin has some affinity for adenosine and guanosine, only a proportion of the nucleoside remains free in the supernatant. Preliminary experiments established that this proportion was independent of the concentration of the nucleosides. The total radioactivity in the substrate at zero time was measured in the supernatant, when all the substrate had been converted into product by addition of commercial phosphodiesterase (10μg). The latter was added to a tube containing 0.15μM substrate in 130μl of the incubation medium. After 15min the reaction was stopped by addition of anion-exchange resin (see above). (Longer periods of incubation often resulted in significant degradation of the products to compounds which bound to the resin.)

Much of the adenosine produced during the phosphodiesterase assay is further converted into inosine, presumably by adenosine deaminase that is present in the tissue homogenate. However, this did not pose a problem in the present study, since it was shown that the proportion of inosine that bound to the resin was identical with that of adenosine.

In some experiments, nucleosides were separated from nucleotides by using a small column of anion-exchange resin (see Loten & Sneyd, 1970; Rutten *et al.*, 1973). No differences in enzyme activities were observed with the two methods. The method described above was preferred because it was less time-consuming and gave greater reproducibility within a given assay.

Calculation of V and K_m for the high- and low-K_m phosphodiesterases

The method used for calculation of V and K_m for the high- and low-K_m phosphodiesterases was a

modification of that of Spears *et al.* (1971). The procedure was as follows. (a) A graph of v against v/s (Hofstee, 1952) was plotted for all the results: the graph frequently appears as two straight-line portions connected by a curve. (b) V and K_m were calculated for the linear portion of the graph at high substrate concentrations. (c) From these values for V and K_m, the ratio, v/s, for the high-K_m enzyme was calculated for the lower substrate concentrations and these values were subtracted from the measured ratio, so that the ratio for the low-K_m enzyme was obtained. (d) From this ratio, the activity due to the low-K_m enzyme was calculated and a new plot of v against v/s was drawn. (e) The linear portion of this graph was used to calculate V and K_m for the low-K_m enzyme. (f) As in stages (c) and (d), the contribution of the low-K_m enzyme to the values of v at the high substrate concentrations was calculated and subtracted from the values for the high-K_m enzyme. A second plot was produced for the high-K_m enzyme, from which more accurate values of V and K_m could be obtained. (g) The cycle (b) to (f) was repeated until the results of the calculations converged to within experimental error of the data. Often two or three cycles were sufficient.

Unfortunately, this procedure was often complicated because the high-K_m enzyme exhibited positive co-operativity in response to substrate concentration, so that the Hofstee plots were curved. In this case, the contribution of the high-K_m enzyme to the values of v/s for the low-K_m enzyme was obtained by extrapolation of the plot to the abscissa. In this work it was assumed that the low-K_m enzyme exhibited no co-operativity.

Expression of results

All enzyme activities are expressed as nmol of product formed/min per g of fresh tissue at 30°C. The activities reported represent the mean of a number of determinations on tissues from different animals. Since, in the present work, no account has been taken of such factors as season, diet, size, age and sex of the animals, and since precise quantitative interpretations are not made from these activities, a conventional statistical presentation of the results was considered undesirable and unnecessary. The numbers of determinations of each activity together with the range of activities are given in parentheses in Tables 2–4. Standard deviations can be calculated from the range of activities. However, any use of the reported activities for precise quantitative analysis must be made with caution.

Results

Control experiments on conditions of extraction and assay

The aim of this comparative study on adenylate cyclase and phosphodiesterase is to provide reliable information on maximum activities and K_m values by which the enzyme from one tissue can be compared with that from another tissue or another animal. One difficulty in such an analysis is the possibility of variation in properties of the enzyme from one tissue and/or animal to another. To eliminate such difficulties, a detailed analysis on the properties of the enzymes from each tissue and each animal should be carried out. However, the amount of work required would restrict the accumulation of data to a very few animals. In the present study, the effects of temperature, extraction conditions and some known activators have been investigated with the enzymes from selected animals representing the major phyla investigated. The conditions of extraction and assay were such as to lower the concentration of inhibitors to insignificant values. The tissues and animals chosen as controls were as follows: cerebral ganglion of the cockroach; flight muscle and cerebral ganglion of the locust; heart and brain of the frog; pectoral muscle and brain of the pigeon; brain, liver and gastrocnemius muscle of the mouse (see Tables 1–4 for systematic names).

Extraction conditions

In the above tissues, the extraction method described in the Materials and Methods section was found to be optimal for both enzymes. For nervous tissue, Triton X-100 (0.1%) increased the activity of adenylate cyclase from the mammals and birds and of phosphodiesterase from all the species investigated by about 1.5–3.0-fold. Higher concentrations of detergent did not lead to any further increase in activity. However, Triton either had no effect or it decreased the activities of both enzymes from muscle or liver and the activities of adenylate cyclase from locust and cockroach cerebral ganglion. Consequently, 0.1% Triton X-100 was included in the extraction medium for phosphodiesterase from all nervous tissues and for adenylate cyclase from vertebrate nervous tissues. There was no effect of sonication of the extracts on the activities of either enzyme. Higher activities of both enzymes were observed when the extraction medium was hypo-osmotic.

Assay conditions

It was decided to perform all assays at the same pH. This was preferred to measurement of activities at the optimal pH for each enzyme, since both enzymes from a number of animals and tissues exhibit broad pH optima between 7.0 and 8.5 (see Perkins, 1973; Appleman *et al.*, 1973). Further, the pH optima for the basal and fluoride-stimulated adenylate cyclase may be different (Kelly & Koritz, 1971). Consequently the enzymes were assayed at a pH that may approximate to the intracellular pH, i.e. 7.5.

To provide enzyme activities that were directly comparable, it was decided to measure the activities of the enzymes from all tissues at 30°C, since many other workers had used this temperature. Since 30°C is above the body temperature of most poikilotherms but below that of most homoiotherms (although the temperature of the thorax of many insects during flight is approx. 30°C and the body temperature of other animals may be greater than 20°C in the summer), the effect of temperature from 10° to 40°C was investigated on enzyme activities from the selected animals (Table 1). In general, the effects of temperature changes were similar for the different enzymes studied. Thus for basal and fluoride-stimulated adenylate cyclase activities, the values of the temperature coefficient, Q_{10} (activities at $T°+10°C$/activities at $T°C$) for the lower temperature ranges were approx. 2–2.7 (although the values for the Q_{10} of the enzyme from the muscle of the mouse were considerably lower). Values of Q_{10} for this enzyme for the 30–40°C temperature range were lower than 2.0, except for the muscle of the mouse. For the phosphodiesterase activities, the values of Q_{10} were usually below 2.0 and they were very low for the temperature range 30–40°C (Table 1). The effect of temperature on the activity of phosphodiesterase was independent of the substrate concentration. Little work on the effect of temperature on these enzymes has been reported: Cheung (1967) and Nair (1966) report a discontinuity in the Arrhenius plot for phosphodiesterase. It is considered that the differences in behaviour of these enzymes with temperature are not sufficient to interfere with the interpretations of the data that are made in the Results and Discussion sections.

Activators

The optimal Mg^{2+} concentration for adenylate cyclase is 5–10mM (Perkins, 1973; Weinryb *et al.*, 1973). Therefore a free concentration of Mg^{2+} (i.e. above the concentration of ATP) of 7mM was used. Although Ca^{2+} inhibits adenylate cyclase, there is evidence that small quantities of Ca^{2+} are necessary for the full expression of its activity (see Perkins, 1973; von Hungen & Roberts, 1973; Johnson & Sutherland, 1973; Poirier *et al.*, 1974; Brostrom *et al.*, 1975; Cheung *et al.*, 1975). Thus a small amount of EDTA (but not EGTA) was included in the present assay medium, although in control experiments it made little difference to the activity of the enzyme. Dithiothreitol was also included in the assay (see Øye & Sutherland, 1966; Rosen *et al.*, 1970; Kelly & Koritz, 1971).

The optimal concentration range of Mg^{2+} for phosphodiesterase activity is 2–10mM (Appleman *et al.*, 1973), so that 6mM-Mg^{2+} was used in the present work. It has been reported that low

Table 1. *Effect of temperature on the basal and fluoride-stimulated activities of adenylate cyclase and cyclic AMP and cyclic GMP phosphodiesterases*

The activities of adenylate cyclase were measured at 0.5mM-ATP. The activities of the phosphodiesterases were measured at concentrations of 1.5, 7.7 and 77μM-cyclic AMP or -cyclic GMP, but since the increments in activity were very similar at each substrate concentration only the data obtained at 7.7μM substrate are presented. Further details of the assays are given in the Materials and Methods section. Activities are presented as means of measurements on extracts from three different animals.

		Enzyme activities (nmol/min per g of fresh tissue)															
		Adenylate cyclase															
		Basal activity				Fluoride-stimulated activity				Cyclic AMP phosphodiesterase				Cyclic GMP phosphodiesterase			
Animal	Temperature (°C) ... Tissue	10	20	30	40	10	20	30	40	10	20	30	40	10	20	30	40
Locust (*Schistocerca gregaria*)	Cerebral ganglion	9.9	22.0	46.0	—	33.0	82.0	179.0	—	218.0	471.0	708.0	—	106.0	185.0	252.0	—
	Flight muscle	—	1.9	4.1	6.9	—	3.2	8.2	18.0	—	86.0	179.0	265.0	—	6.3	9.7	9.8
Frog (*Rana temporaria*)	Brain	2.5	6.1	13.0	—	3.5	7.8	18.0	—	71.0	140.0	146.0	—	228.0	319.0	452.0	—
	Heart	0.5	1.1	3.3	—	0.9	1.8	5.3	—	40.0	60.0	79.0	—	91.0	105.0	137.0	—
Domestic pigeon (*Columba livia*)	Brain	—	37.0	101.0	146.0	—	61.0	163.0	221.0	—	298.0	487.0	493.0	—	896.0	1810.0	1840.0
	Pectoral muscle	—	0.5	0.8	1.5	—	1.4	2.6	6.1	—	14.0	27.0	58.0	—	26.0	46.0	82.0
Laboratory mouse	Brain	—	68.0	130.0	182.0	—	87.0	182.0	273.0	—	475.0	537.0	655.0	—	1710.0	2510.0	2560.0
	Liver	—	1.4	2.6	3.9	—	4.2	6.7	11.0	—	26.0	43.0	56.0	—	91.0	169.0	225.0
	Gastrocnemius muscle	—	0.5	0.7	1.5	—	1.3	1.8	4.2	—	10.0	16.0	27.0	—	8.7	13.0	14.0

Table 2. *Basal and fluoride-stimulated activities of adenylate cyclase, and maximal activities of cyclic AMP and cyclic GMP phosphodiesterases in muscles from vertebrates and invertebrates*

The enzyme assays and the calculation of the activities of the phosphodiesterases are described in the Materials and Methods section. Activities are presented as mean V values, with the range of activities and the number of animals used given in parentheses. If the kinetics indicated that only one phosphodiesterase was present, and if the K_m was below 5μM, the enzyme is classified as 'low-K_m': * indicates that the activity of adenylate cyclase or phosphodiesterase was measured at a substrate concentration of 250μM; † for the estimation of the limit of detectable phosphodiesterase activity it was assumed that the ratio of the K_m values for the low- and high-K_m enzymes was 10, since the limit depends on the relative activities and the kinetics of the two enzymes.

		Enzyme activities (nmol/min per g of fresh muscle)					
		Adenylate cyclase		Cyclic AMP phosphodiesterase		Cyclic GMP phosphodiesterase	
Animal	Muscle	Basal activity	Fluoride-stimulated activity	Low-K_m	High-K_m	Low-K_m	High-K_m
Scallop (*Pecten maximus*)	Fast adductor	0.2 (0.2) (2)	—	34 (27–41)	143 (71–215) (2)	—	—
	Slow adductor	3.5 (1.1–5.9) (2)	—	393 (297–490)	660 (390–930) (2)	—	—
Lobster (*Homarus vulgaris*)	Abdomen	0.4 (0.3–0.5) (2)	—	8.0 (4.1–12)	6.3 (4.0–8.6) (4)	0.7 (0.4–1.0)	3.6 (3.0–4.3) (2)
	Chela	0.5 (0.5) (2)	—	5.2 (4.3–6.1)	21 (13–29) (2)	0.6 (0.4–0.7)	4.7 (3.0–6.4) (2)
Locust (*Schistocerca gregaria*)	Flight	6.3 (4.4–8.1) (7)	10.1 (8.6–11) (4)	116 (86–134)	159 (84–209) (3)	11 (8–12)	52 (42–64) (3)
	Hind-leg femoral	0.9 (0.7–1.1) (4)	3.4 (2.3–4.5) (2)	20 (17–23)	75 (67–88) (3)	4.9 (3.2–6.6)	34 (31–36) (3)
Cockroach (*Blaberus discoidalis*)	Flight	5.7 (4.8–7.4) (3)	—	131 (105–172)	83 (57–131) (3)	5.5 (4.4–6.8)	22 (20–23) (3)
Rosechafer (*Pachnoda ephippiata*)	Flight	2.8 (2.6–3.3) (3)	—	70 (51–91)	<30† (4)	1.1 (1.0–1.5)	14* (11–17) (3)
Honey-bee (*Apis mellifera*)	Flight	0.7 (0.4–1.0) (3)	3.1 (1.5–4.0) (3)	39 (24–53)	<15† (4)	3.4 (2.0–4.6)	22 (18–30) (3)
Bumble-bee worker (*Bombus* sp.)	Flight	4.3 (3.0–6.9) (3)	9.3 (5.3–12) (3)	35 (32–39)	57 (41–75) (3)	—	—
Blowfly (*Calliphora erythroencephala*)	Flight	5.2 (4.2–6.5) (3)	—	53 (43–64)	123 (100–145) (4)	<1.0†	12.4 (8.3–20) (4)
Rainbow trout (*Salmo gairdneri*)	Heart	2.0 (1.7–2.3) (2)	8.8 (8.1–9.5) (2)	107 (103–111)	102 (75–130) (2)	55 (53–57)	192 (164–220) (2)
	Red abdominal	1.1 (0.9–1.3) (2)	—	5.6 (4.3–6.9)	28 (20–36) (2)	2.4 (1.3–2.5)	43 (34–52) (2)
	White abdominal	<0.3 (3)	1.8 (1.7–2.0) (3)	1.5 (1.2–1.8)	6.3 (5.1–7.5) (2)	0.3 (0.3)	7.7 (6.7–8.8) (2)
Frog (*Rana temporaria*)	Heart	3.2 (1.9–4.6) (5)	6.5 (3.7–10.2) (5)	58 (53–65)	1150 (1030–1360)(3)	43 (25–58)	770 (604–938) (2)
	Gastrocnemius	0.9 (0.5–1.3) (4)	1.4 (1.3–1.5) (2)	6.0 (3.2–7.0)	43 (32–49) (3)	2.1 (1.5–2.7)	43 (25–59) (2)

Domestic pigeon (*Columba livia*)	Heart	16 (9.1–25) (7)	44 (26–64) (4)	308 (205–410)	875 (750–1000) (2)	331 (259–377)	385 (371–413) (3)
	Pectoral	1.4 (1.0–2.6) (8)	5.6 (4.2–6.9) (4)	13 (12–14)	130 (66–240) (4)	<1.0†	243 (178–307) (3)
	Sartorius	0.5 (0.4–0.6) (3)	1.9 (1.6–2.2) (3)	—	—	—	—
Domestic fowl (*Gallus gallus*)	Heart	3.8 (2.7–4.8) (6)	7.3 (5.4–7.5) (3)	71 (61–85)	167 (74–217) (3)	50 (38–57)	124 (115–129) (3)
	Pectoral	0.5 (0.3–0.7) (6)	0.9 (0.8–0.9) (3)	8.6 (8.5–8.7)	13 (11–16) (3)	2.7 (2.2–3.4)	19 (16–21) (3)
	Sartorius	0.3 (0.3–0.4) (3)	1.1 (1.1) (2)	9.0 (7.9–11)	11 (9–13) (2)	16 (11–21)	13 (12–15) (3)
Laboratory mouse	Heart	6.7 (5.9–9.5) (5)	18 (17–19) (3)	30 (20–39)	341 (323–352) (3)	<2.0†	448 (429–476) (3)
	Gastrocnemius	1.1 (0.7–1.4) (6)	2.7 (2.0–3.5) (4)	10.7 (9.8–12.6)	51 (40–77) (3)	<1.0†	44 (30–54) (3)
Laboratory rat	Heart	11.8 (9.0–17) (9)	25 (16–35) (5)	12 (11–15)	226 (151–277) (4)	<2.0†	296 (272–320) (3)
	Gastrocnemius	0.9 (0.6–1.4) (3)	2.6 (1.9–2.9) (3)	15 (11–18)	70 (67–74) (4)	<1.0†	46 (30–53) (4)

concentrations of Ca^{2+} stimulate partially purified, EGTA-treated phosphodiesterase (see Appleman *et al.*, 1973; Kakiuchi *et al.*, 1975); the maximal effect occurs between 2 and 10μM-Ca^{2+}. Since this concentration of Ca^{2+} would be present in the incubation medium (owing to the Ca^{2+} present in the tissue homogenate) and since high concentrations of Ca^{2+} inhibit the enzyme (see Lagarde & Colobert, 1972), this ion was not added to the assay medium. Dithiothreitol was included in the assay medium (see Cheung & Jenkins, 1969).

Activities of adenylate cyclase and phosphodiesterase

The mean basal activity of adenylate cyclase in muscle tissue ranges from 0.2 to 16nmol/min per g fresh wt. of tissue (fast adductor of scallop and cardiac muscle of pigeon respectively) (Table 2); the fluoride-stimulated activity ranges from 0.9 to 44nmol/min per g (pectoral muscle of domestic fowl and cardiac muscle of pigeon respectively). The total cyclic AMP phosphodiesterase activity (i.e. low-K_m plus high-K_m enzymes) ranges from 7.8 to 1180nmol/min per g (white abdominal muscle of trout and cardiac muscle of pigeon respectively). The total cyclic GMP phosphodiesterase activity ranges from 4.3 to 816nmol/min per g (abdominal muscle of lobster and cardiac muscle of pigeon respectively).

There is a correlation between the activity of adenylate cyclase in a muscle and its capacity for oxidative metabolism (see Table 2). This correlation has been noted previously on the basis of some activities for vertebrate muscles (Severson *et al.*, 1972). Thus the highest basal or fluoride-stimulated activities of adenylate cyclase were observed in insect flight muscles and vertebrate hearts, lower activities were observed in aerobic (red) vertebrate skeletal muscles and the lowest activities were observed in the anaerobic (white) muscles. Although similar correlations exist for the total activities of cyclic AMP and cyclic GMP phosphodiesterase, they are less distinct. For example, the total activities of cyclic GMP phosphodiesterase from insect flight muscle are low; they are similar to the activities of the enzyme from vertebrate skeletal muscle.

The activities of these enzymes in the livers of vertebrates are presented in Table 3. The mean basal activity of adenylate cyclase ranges from <0.5 to 5.5nmol/min per g (rainbow trout and rat respectively): the fluoride-stimulated activity ranges from 2.9 to 17nmol/min per g (rainbow trout and rat respectively). The total cyclic AMP phosphodiesterase activity ranges from 58 to 743nmol/min per g (rainbow trout and mouse respectively). The total cyclic GMP phosphodiesterase activity ranges from 78 to 840nmol/min per g (rainbow trout and mouse respectively). The activities of these enzymes

Table 3. *Basal and fluoride-stimulated activities of adenylate cyclase and maximal activities of cyclic AMP and cyclic GMP phosphodiesterases in livers from vertebrates*

See legend to Table 2 for details of presentation and interpretation of †.

	Enzyme activities (nmol/min per g of fresh liver)					
	Adenylate cyclase		Cyclic AMP phosphodiesterase		Cyclic GMP phosphodiesterase	
Animal	Basal activity	Fluoride-stimulated activity	Low-K_m	High-K_m	Low-K_m	High-K_m
Rainbow trout (*Salmo gairdneri*)	<0.5 (3)	2.9 (2.1–3.9) (3)	18 (11–25)	40 (39–41) (2)	8.0 (7.0–8.9) (2)	70 (58–82) (2)
Frog (*Rana temporaria*)	3.8 (3.0–4.5) (4)	13 (13–14) (2)	12 (8–17)	263 (200–340) (3)	22 (20–25)	248 (216–283) (3)
Domestic pigeon (*Columba livia*)	2.8 (1.9–4.8) (5)	7.6 (6.1–9.1) (2)	19 (18–19)	366 (335–426) (3)	—	—
Domestic fowl (*Gallus gallus*)	1.3 (0.8–1.7) (4)	2.9 (2.5–3.2) (2)	5.0 (3.8–6.4)	74 (68–80) (3)	<0.5†	114 (103–126) (3)
Laboratory mouse	3.4 (2.4–4.2) (6)	9.5 (7.9–11) (6)	23 (14–29)	720 (560–1030) (3)	<4.0†	848 (682–1040) (3)
Laboratory rat	5.5 (3.6–8.2) (3)	17 (14–18) (4)	19 (15–25)	481 (380–567) (4)	<3.0†	588 (444–691) (4)

in liver are somewhat similar to the activities in hearts of the same animals.

In nervous tissue the activities of adenylate cyclase and, in some species, high-K_m phosphodiesterase were exceptionally high (Table 4). The mean basal activity of adenylate cyclase ranges from 3 to 240nmol/min per g (brain of the lobster and rat respectively): the fluoride-stimulated activity ranges from 28 to 320nmol/min per g (brain of the frog and rat respectively). The total cyclic AMP phosphodiesterase activity ranges from 1160 to 10200nmol/min per g (cerebral ganglion of the cockroach and brain of the domestic pigeon respectively). The total cyclic GMP phosphodiesterase activity ranges from 385 to 10300nmol/min per g (cerebral ganglion of lobster and brain of domestic fowl respectively).

The activities of adenylate cyclase from livers and the fluoride-stimulated activities from nervous tissues varied less than those from muscle. In vertebrate livers, the activities of the low-K_m cyclic AMP phosphodiesterase were all similar. The low-K_m enzyme was consistently detected in homogenates of rat liver. This finding differs from that of Thompson & Appleman (1971), who found that the low-K_m enzyme was only detectable after homogenates (or sonicated 100000g supernatant preparations of rat liver) had been kept at 4°C for 24h (Russell *et al.*, 1973). In the present study, the kinetics of cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase were identical when the enzymes were extracted at 30°C and assayed immediately or when they were extracted at 4°C and assayed after the homogenate had been incubated for 2h at this temperature.

Stimulation of the activity of adenylate cyclase by fluoride

In most tissues, the activity of adenylate cyclase was increased 2–4-fold by fluoride (Table 5), but there was a consistently lower effect on the activity of the enzyme from vertebrate brain, in agreement with previous observations (Sutherland *et al.*, 1962). Similar degrees of stimulation by fluoride (and hormones) of adenylate cyclase activities in crude homogenates have been observed in other studies (see, for example, Murad & Vaughan, 1969; Birnbaumer *et al.*, 1970; Triner *et al.*, 1971; Severson *et al.*, 1972). However, in some experiments larger effects of fluoride (up to 31-fold increases in enzyme activity) have been observed with purified or fractionated preparations of adenylate cyclase (see Perkins, 1973; Drummond & Duncan, 1970).

Values of K_m and deviations from Michaelis–Menten kinetics

The K_m values obtained for adenylate cyclase and the phosphodiesterases are presented in Table 6. Phosphodiesterase exhibits kinetics that have been interpreted as being due to the presence of 'low-K_m' and 'high-K_m' enzymes. Where applicable, the K_m values reported here are similar to previously reported values (see Appleman *et al.*, 1973; Rall, 1969). The present results indicate a remarkable consistency in the K_m values from different tissues and different animals. The K_m value of adenylate cyclase for ATP ranges from 100 to 660μM (cardiac muscle of the domestic fowl and liver of the frog respectively). The K_m for the low-K_m cyclic AMP phosphodiesterase ranges from 0.5 to 4.1μM (brain

Table 4. *Basal and fluoride-stimulated activities of adenylate cyclase and maximal activities of cyclic AMP and cyclic GMP phosphodiesterases in nervous tissues from vertebrates and invertebrates*

See legend to Table 2 for details of presentation and interpretation of * and †.

Animal	Nervous tissue	Enzyme activities (nmol/min per g of fresh tissue)					
		Adenylate cyclase		Cyclic AMP phosphodiesterase		Cyclic GMP phosphodiesterase	
		Basal activity	Fluoride-stimulated activity	Low-K_m	High-K_m	Low-K_m	High-K_m
Lobster (*Homarus vulgaris*)	Cerebral ganglion	3.0 (2.2–3.8) (3)	—	101 (88–120)	180* (140–250) (3)	130 (110–140)	255 (230–280) (3)
Locust (*Schistocerca gregaria*)	Cerebral ganglion	54 (42–67) (8)	171 (167–176) (4)	395 (320–520)	1690 (1000–2080) (3)	65 (50–76)	3730 (3500–3950) (3)
Cockroach (*Blaberus discoidalis*)	Cerebral ganglion	63 (44–80) (6)	200 (185–210) (3)	770 (730–800)	390 (370–400) (3)	525 (410–710)	1370 (1180–1560) (3)
Rosechafer (*Pachnoda ephippiata*)	Cerebral ganglion	38 (25–53) (2)	—	225 (195–250)	226* (184–293) (3)	41 (38–44)	131* (123–137) (3)
Frog (*Rana temporaria*)	Whole brain	20 (14–25) (5)	28 (19–37) (4)	31 (25–39)	1190 (990–1500) (3)	<5.0†	1170 (1090–1240) (3)
Domestic pigeon (*Columba livia*)	Whole brain	149 (94–223) (5)	247 (211–298) (3)	61 (54–94)	10100 (9000–11600) (3)	—	—
Domestic fowl (*Gallus gallus*)	Whole brain	160 (130–170) (5)	241 (215–267) (3)	79 (33–115)	8470 (7700–8950) (3)	<40†	10300 (8950–11800) (3)
Laboratory mouse	Whole brain	154 (110–205) (5)	214 (160–240) (4)	95 (82–130)	5550 (5520–5600) (3)	<30†	6930 (5980–8250) (3)
Laboratory rat	Whole brain	240 (210–315) (3)	320 (230–449) (4)	56 (38–88)	6660 (5000–9920) (3)	<20†	5470 (3350–6700) (3)

Table 5. *Stimulation of the activities of adenylate cyclase from different tissues and animals by fluoride*

Experimental details are given in the Materials and Methods section. The ratio activity in the presence of fluoride/activity in the absence of fluoride, is the mean ratio obtained from a number of determinations of the ratio for each tissue (i.e. it is not simply the ratio of the mean activities given in Tables 2, 3 and 4). The ranges and the number of determinations of the ratio are given in parentheses. See Tables 2–4 for systematic names.

Animal	Tissue	Fluoride-stimulated adenylate cyclase activity / Basal adenylate cyclase activity
Locust	Cerebral ganglion	3.8 (3.4–4.2) (4)
	Flight muscle	1.7 (1.6–2.0) (4)
	Leg muscle	3.3 (2.4–4.1) (3)
Cockroach	Cerebral ganglion	3.4 (2.8–4.2) (3)
Honey-bee	Flight muscle	4.1 (3.3–5.3) (3)
Bumble-bee	Flight muscle	2.5 (1.8–3.7) (3)
Frog	Brain	1.4 (1.3–1.5) (4)
	Liver	3.5 (3.5–3.6) (2)
	Heart	1.9 (1.5–2.3) (6)
	Gastrocnemius muscle	2.1 (1.9–2.3) (2)
Pigeon	Brain	1.7 (1.3–2.3) (3)
	Liver	3.9 (3.8–3.9) (2)
	Heart	2.7 (2.2–3.1) (4)
	Pectoral muscle	3.5 (2.6–4.0) (4)
	Sartorius muscle	3.9 (3.8–4.1) (2)
Domestic fowl	Brain	1.6 (1.4–1.7) (2)
	Liver	3.1 (2.8–3.3) (2)
	Heart	2.0 (2.0) (3)
	Pectoral muscle	2.0 (1.7–2.2) (3)
	Sartorius muscle	3.7 (3.6–3.9) (2)
Mouse	Brain	1.4 (1.3–1.4) (4)
	Liver	3.0 (2.0–3.8) (6)
	Heart	2.6 (2.1–2.8) (3)
	Gastrocnemius muscle	2.9 (2.0–3.5) (3)
Rat	Brain	1.3 (1.1–1.6) (5)
	Liver	3.2 (2.2–3.9) (3)
	Heart	2.0 (1.8–2.1) (5)
	Gastrocnemius muscle	2.9 (2.2–3.6) (3)

of the mouse and slow-adductor muscle of the scallop respectively); the K_m for the high-K_m enzyme ranges from 15 to >200μM (flight muscle of the bumble-bee and several other tissues respectively). The K_m for the low-K_m cyclic GMP phosphodiesterase ranges from 0.5 to 6.3μM (cerebral ganglion of the locust and cerebral ganglion of the cockroach respectively). The K_m for the high-K_m enzyme ranges from 12 to >200μM (cardiac muscle of the pigeon and several other tissues respectively). (The K_m of 7.1μM measured for flight muscle of the blowfly could be classified as either low- or high-K_m.)

The high-K_m phosphodiesterase frequently exhibited kinetics which were non-linear in the Hofstee plot and corresponded to sigmoid kinetics in a plot of activity against substrate concentration. Similar observations have been made for partially purified phosphodiesterases (Beavo *et al.*, 1970; Scott & Solomon, 1973; Bevers *et al.*, 1974; Sakai *et al.*, 1974; Teresaki & Appleman, 1975; Appleman & Teresaki, 1975). However, the value of the Hill coefficient for the high-K_m phosphodiesterase rarely exceeded 1.2 for the range of substrate concentrations near the K_m (see Teresaki & Appleman, 1975). The highest value of the Hill coefficient was 2.0, which was observed for cyclic AMP diesterase from rat liver.

In the above discussions it has been assumed that the kinetics of phosphodiesterase, observed in crude extracts, result from the presence of two enzymes (i.e high- and low-K_m enzymes). Further in most tissues the plots of activity against substrate concentration for the individual enzymes were either hyperbolic or sigmoid. However, in some cases, these plots were such as to indicate negative co-operativity or the presence of more than one enzyme (i.e. more than two phosphodiesterases). Thus the high-K_m cyclic GMP phosphodiesterase

Table 6. *K_m values of the adenylate cyclases for ATP, cyclic AMP phosphodiesterases for cyclic AMP and cyclic GMP phosphodiesterases for cyclic GMP*

The measurement of the K_m values and the method used to calculate the K_m values for the phosphodiesterase enzymes are described in the Materials and Methods section. The K_m values presented were calculated from the linear portion of a Hofstee plot. However, in some tissues the Hofstee plots for the high-K_m phosphodiesterase deviated so markedly from linear that K_m values could not be obtained: these are indicated by an asterisk. The K_m values of phosphodiesterase are those obtained from a substrate concentration range, 0.15 to 500μM. The K_m values of adenylate cyclase were measured in the absence of fluoride. Mean values are presented, with the range and number of animals used given in parentheses. See Tables 2, 3 or 4 for systematic names.

		K_m (μM)				
			Cyclic AMP phosphodiesterase		Cyclic GMP phosphodiesterase	
Animal	Tissue	Adenylate cyclase	Low-K_m	High-K_m	Low-K_m	High-K_m
Scallop	Fast-adductor muscle	200 (150–260) (2)	2.5 (2.3–2.6)	90 (69–109) (2)	—	—
	Slow-adductor muscle	280 (280) (2)	4.1 (3.2–4.9)	91 (75–107) (2)	—	—
Lobster	Cerebral ganglion	—	1.3 (0.6–2.1)	>200 (3)	3.0 (1.9–4.1)	100 (40–150) (3)
	Abdominal muscle	—	2.4 (1.2–3.1)	120 (50–200) (4)	1.9 (0.9–2.9)	72 (52–93) (2)
	Claw muscle	—	1.3 (1.3–1.4)	140 (140–150) (2)	2.5 (2.0–3.0)	80 (40–120) (2)
Locust	Cerebral ganglion	170 (140–200) (3)	0.8 (0.6–1.1)	77 (66–89) (3)	0.5 (0.4–0.7)	79 (68–96) (3)
	Flight muscle	—	1.5 (0.7–2.8)	24 (20–25) (3)	4.6 (4.4–4.7)	59 (40–76) (3)
	Hind-leg muscle	210 (110–300) (3)	1.4 (0.8–2.0)	34 (30–37) (3)	2.0 (1.7–2.4)	70 (43–107) (3)
Cockroach	Cerebral ganglion	180 (160–200) (3)	1.6 (1.6)	29 (15–40) (3)	6.2 (4.3–8.2)	73 (60–80) (3)
	Flight muscle	170 (160–180) (2)	1.8 (1.2–2.1)	43 (25–60) (3)	1.9 (1.5–2.0)	61 (38–78) (3)
Rosechafer	Cerebral ganglion	170 (150–200) (2)	1.5 (1.1–1.9)	>200 (3)	5.0 (4.7–5.4)	>200 (3)
	Flight muscle	320 (240–390) (3)	1.7 (1.6–1.8)	— (3)	1.6 (1.5–1.8)	>200 (3)
Honey-bee	Flight muscle	—	1.9 (1.5–2.5)	— (4)	1.0 (0.6–1.3)	67 (45–95) (3)
Bumble-bee	Flight muscle	—	1.8 (1.0–2.8)	15 (6–20) (3)	—	—
Blowfly	Flight muscle	370 (320–420) (3)	1.7 (1.7–1.8)	35 (31–39) (3)	—	71 (5.1–9.3) (3)
Rainbow trout	Brain	250 (100–350) (3)	—	—	—	—
	Liver	—	2.0 (1.1–2.9)	38 (27–48) (2)	1.3 (1.3–1.4)	44 (39–49) (2)
	Heart	—	2.3 (1.7–2.8)	— (2)	0.8 (0.8–0.9)	83 (83) (2)
	Red abdominal muscle	—	2.7 (2.4–3.0)	50 (41–60) (2)	2.3 (2.1–2.6)	44 (41–47) (2)
	White abdominal muscle	—	1.8 (1.6–2.0)	78 (50–87) (2)	2.0 (1.9–2.1)	70 (67–73) (2)
Frog	Brain	—	0.9 (0.7–1.1)	* (3)	—	20 (13–27) (3)
	Liver	660 (518–820) (3)	0.8 (0.5–1.1)	136 (133–140) (3)	4.7 (4.2–5.2)	109 (85–133) (3)
	Heart	120 (70–190) (3)	0.9 (0.8–1.0)	183 (170–200) (3)	2.0 (1.8–2.1)	47 (28–57) (3)
	Gastrocnemius muscle	500 (260–790) (3)	1.7 (1.0–2.1)	250 (135–400) (3)	2.3 (2.3–2.4) (3)	76 (73–79) (3)

Table 6—*continued*

		K_m (μM)				
			Cyclic AMP phosphodiesterase		Cyclic GMP phosphodiesterase	
Animal	Tissue	Adenylate cyclase	Low-K_m	High-K_m	Low-K_m	High-K_m
Domestic pigeon	Brain	290	0.6	*	—	—
		(180–360) (5)	(0.6–0.7)	(3)		
	Liver	210	0.7	*	—	—
		(200–220) (3)	(0.5–1.0)	(3)		
	Heart	180	1.4	78	1.3	12
		(160–220) (4)	(1.3–1.4)	(76–81) (2)	(1.2–1.5)	(11–16) (3)
	Pectoral muscle	360	0.8	95	—	37
		(300–500) (3)	(0.66–0.92)	(50–140) (3)		(32–45) (3)
Domestic fowl	Brain	170	1.0	46	—	58
		(110–200) (4)	(0.75–1.5)	(35–69) (3)		(43–67) (3)
	Liver	160	0.7	47	—	28
		(120–220) (3)	(0.48–0.85)	(32–73) (3)		(22–32) (3)
	Heart	100	1.3	38	2.1	19
		(60–160) (3)	(1.2–1.3)	(21–56) (3)	(1.7–2.5)	(13–23) (3)
	Pectoral muscle	610	1.7	22	—	—
		(300–1100) (3)	(1.5–1.7)	(21–25) (3)		
	Sartorius muscle	—	0.8	23	—	—
			(0.7–0.9)	(20–25) (2)		
Laboratory mouse	Brain	170	0.5	68	—	17
		(80–250) (6)	(0.2–0.9)	(53–81) (3)		(16–18) (3)
	Liver	230	1.1	58	—	32
		(120–370) (4)	(0.7–1.4)	(43–80) (3)		(30–34) (3)
	Heart	130	1.7	70	—	24
		(70–200) (3)	(1.4–2.0)	(58–78) (3)		(22–25)(3)
	Gastrocnemius muscle	410	1.6	41	—	21
		(320–470) (3)	(1.3–2.0)	(31–52) (5)		(13–29) (3)
Laboratory rat	Brain	—	0.3	42	—	21
			(0.2–0.4)	(24–56) (3)		(25) (3)
	Liver	—	0.7	*	—	24
			(0.4–1.2)	(4)		(17–33) (4)
	Heart	110	0.7	40	—	18
		(80–160) (13)	(0.4–1.0)	(28–50) (4)		(15–22) (3)
	Gastrocnemius muscle	—	1.7	51	—	21
			(1.5–1.9)	(47–56) (3)		(15–27) (4)

from pigeon liver has an apparent Hill coefficient of 0.83 and the low-K_m enzyme from rat liver has a coefficient of 0.95.

Discussion

The present work has shown that high activities of adenylate cyclase and cyclic AMP phosphodiesterase are present in nervous tissues and cardiac muscles from a wide range of animals (Tables 2 and 4). Explanations for high activities in these tissues are given later in this section. Intermediate activities of adenylate cyclase are found in aerobic skeletal muscles (including both vertebrate and insect flight muscles), whereas low activities are found in the more anaerobic muscles (Table 2). It has been suggested that higher activities are present in red muscle because it is more dependent on lipolysis and subsequent fatty acid oxidation for energy formation than is white muscle (see Severson *et al.*, 1972). However, there is some evidence to suggest that endogenous triacylglycerol in muscle is an unimportant source of energy compared with that supplied by the oxidation of exogenous fatty acids (Masoro *et al.*, 1966; Masoro, 1967; Carlson, 1969). In addition, the present paper reports high activities of adenylate cyclase in insect flight muscles that do not utilize fat as a source of energy (e.g. cockroach, honey-bee, bumble-bee, blowfly; see Crabtree & Newsholme, 1972). It is suggested that one possible role of cyclic AMP in these muscles (and perhaps in heart muscle) is the control of a mitochondrial process (e.g. Ca^{2+} translocation; see Borle, 1974; Matlib & O'Brien, 1974; Rasmussen *et al.*, 1975).

Table 7. *Ratios of the maximal activities of cyclic AMP and cyclic GMP phosphodiesterases from different tissues and animals*

Data are taken from Tables 2–4. Ratios of zero and infinity indicate that the activities of cyclic GMP phosphodiesterase and cyclic AMP phosphodiesterase respectively were not detectable.

		Activity of cyclic GMP phosphodiesterase/Activity of cyclic AMP phosphodiesterase	
Animal	Tissue	Low-K_m enzyme	High-K_m enzyme
Lobster	Cerebral ganglion	1.29	—
	Abdominal muscle	0.09	0.57
	Claw muscle	0.12	0.22
Locust	Cerebral ganglion	0.16	2.21
	Flight muscle	0.09	0.33
	Hind-leg muscle	0.25	0.45
Cockroach	Cerebral ganglion	0.68	3.51
	Flight muscle	0.04	0.27
Rosechafer	Cerebral ganglion	0.18	—
	Flight muscle	0.02	∞
Honey-bee	Flight muscle	0.09	∞
Blowfly	Flight muscle	0.0	0.10
Trout	Liver	0.44	1.75
	Heart	0.19	1.88
	Red abdominal muscle	0.43	1.53
	White abdominal muscle	0.20	1.22
Frog	Brain	0.0	0.98
	Liver	1.83	0.94
	Heart	0.74	0.67
	Gastrocnemius muscle	0.35	1.00
Pigeon	Heart	1.07	0.44
	Pectoral muscle	0.0	1.87
Domestic fowl	Brain	0.0	1.22
	Liver	0.0	1.54
	Heart	0.70	0.74
	Pectoral muscle	0.31	1.46
	Sartorius muscle	1.78	1.18
Mouse	Brain	0.0	1.25
	Liver	0.0	1.18
	Heart	0.0	1.31
	Gastrocnemius muscle	0.0	0.86
Rat	Brain	0.0	0.82
	Liver	0.0	1.22
	Heart	0.0	1.31
	Gastrocnemius muscle	0.0	0.66

Cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase

The ratio activity of high-K_m cyclic GMP phosphodiesterase/activity of high-K_m cyclic AMP phosphodiesterase shows considerable variation for the invertebrate tissues, but the variation is much less for the vertebrate tissues (see Table 7) (see also Beavo *et al.*, 1970; Robison *et al.*, 1971, p. 406). These results suggest that in invertebrate tissues there are at least two separate cyclic nucleotide phosphodiesterases, one specific for cyclic AMP and the other specific for cyclic GMP, whereas in vertebrate tissues there is only one enzyme with dual specificity (see also Appleman & Teresaki, 1975).

The high-K_m phosphodiesterase from vertebrate tissues has a K_m for cyclic GMP which is as much as 3-fold lower than that for cyclic AMP (Table 6). The greater affinity for cyclic GMP does not indicate that the enzyme specifically hydrolyses cyclic GMP (i.e. it is a 'functional' cyclic GMP phosphodiesterase), because the values of V for the two substrates are similar and the concentration of cyclic GMP in the cell is usually much lower than that of cyclic AMP (at least 10-fold; see Goldberg *et al.*, 1973) (see also Teresaki & Appleman, 1975).

The ratio, activity of low-K_m GMP phosphodiesterase/activity of low-K_m cyclic AMP phosphodiesterase, shows considerable variation between the tissues of all the animals investigated (Table 7). These results suggest that, among both vertebrate and invertebrate tissues, there is more than one

low-K_m enzyme for the hydrolysis of these cyclic nucleotides.

Simple model to explain the steady-state concentration of cyclic AMP and changes in this concentration in vivo

Changes in the cyclic AMP concentration are important as intracellular regulators for many biochemical and physiological systems in living organisms. It is therefore important to understand the biochemical mechanism that produces changes in this concentration. A simple enzymic model has been developed to account for both the maintenance of the steady-state concentration of cyclic AMP and the changes in its concentration from one steady state to another. The results obtained in the present study are examined by using this model.

The model is a quantitative extension of that discussed by Newsholme & Start (1973) and a more detailed model has been discussed by Davies & Williams (1975). The following assumptions are implicit in the model: that cyclic AMP production occurs only via adenylate cyclase and that degradation of the nucleotide occurs only via the activities of the high- and low-K_m phosphodiesterases; that adenylate cyclase is saturated with substrate [this is a reasonable assumption, since the K_m of the cyclase from a number of tissues from many different animals is about 0.2mM (Table 6) and in most tissues the ATP content is at least 2μmol/g fresh wt. of tissue (see Beis & Newsholme, 1975)]; and that all three enzymes catalyse non-equilibrium reactions *in vivo*. Therefore the following reaction sequences describe the model:

The activity of adenylate cyclase is represented by a, that of the low-K_m phosphodiesterase by l, and that of the high-K_m phosphodiesterase by h. Thus in the steady-state situation, a = h+l.

If it is further assumed that both phosphodiesterase enzymes obey Michaelis–Menten kinetics, the above equation extends to:

$$V_a = \frac{V_l s}{K_{ml}+s} + \frac{V_h s}{K_{mh}+s}$$

where s is the concentration of cyclic AMP and the subscripts a, l and h refer to the enzymes as indicated above. From this equation the steady-state concentration of cyclic AMP in any tissue can be computed from the value of V for adenylate cyclase and the values of K_m and V for the low- and high-K_m phosphodiesterases for that tissue (see below). Further, the model can be extended to predict the changes in cyclic AMP concentration produced by the maximal effect of hormones or other agents on adenylate cyclase. This extension assumes that the basal activity of adenylate cyclase *in vitro* (see the Materials and Methods section) represents the basal activity of the enzyme *in vivo* (i.e. in the absence of hormonal stimulation) and that fluoride-stimulated activity (*in vitro*) represents the maximal stimulation by hormones. This is a reasonable assumption, since the activities of adenylate cyclase from most tissues are not responsive to hormones in the presence of maximally effective concentrations of fluoride; see Perkins (1973). However, glucagon is an exception, since liver adenylate cyclase activity is stimulated to a greater extent by glucagon than by fluoride; see Birnbaumer *et al.* (1971) and Pilkis *et al.* (1974). The basal concentration of cyclic AMP is obtained by insertion of the basal adenylate cyclase activity into the above equation and the stimulated concentration by insertion of the fluoride-stimulated activity. An example of this calculation, for locust flight muscle (data from Tables 2 and 6) is as follows.

The basal concentration of cyclic AMP (s_b) is obtained from the equation:

$$6.3 = \frac{116s}{1.5+s} + \frac{159s}{24+s}$$

so that $s_b = 0.08\,\mu$M. The stimulated concentration of cyclic AMP (s_s) is obtained from the equation:

$$10.1 = \frac{116s}{1.5+s} + \frac{159s}{24+s}$$

so that $s_s = 0.13\,\mu$M.

Unfortunately, this simple calculation cannot be used for many tissues, since the high-K_m phosphodiesterase does not obey Michaelis–Menten kinetics (see above). However, since under steady-state conditions the activity of adenylate cyclase equals the total (low-K_m plus high-K_m) activity of phosphodiesterase, the latter is used to calculate the cyclic AMP concentrations. Hence, reference to the plot of phosphodiesterase activity against cyclic AMP concentration indicates the cyclic AMP concentrations provided by the activity of adenylate cyclase (since adenylate cyclase activity equals that of phosphodiesterase). For the example calculated above, this latter treatment gives mean values of s_b and s_s of 0.08 and 0.14μM respectively, which are very similar to those values calculated above.

The concentrations of cyclic AMP under basal and stimulated conditions have been calculated from the mean activities of adenylate cyclase in Tables 2–4 and the individual plots of phosphodiesterase (from which the results in Tables 2–4 have been obtained): these concentrations are presented in Table 8. The ratio stimulated concentration/basal concentration for cyclic AMP ranges from 1.7 to 7.4 (the basal concentrations range from 0.01 to 2.2μM and the

Table 8. *Concentrations of cyclic AMP calculated from a model of cyclic AMP turnover*

The calculation of the basal and stimulated steady-state concentrations of cyclic AMP is described in the text. The concentrations are calculated from the mean activities of the enzyme given in Tables 2–4.

Animal	Tissue	Calculated concentrations of cyclic AMP (μM) Basal conditions	Calculated concentrations of cyclic AMP (μM) Stimulated conditions	Ratio of concentrations (basal/stimulated)
Scallop	Fast-adductor muscle	0.01	—	—
	Slow-adductor muscle	0.04	—	—
Lobster	Cerebral ganglion	0.06	—	—
	Abdominal muscle	0.27	—	—
	Claw muscle	0.11	—	—
Locust	Cerebral ganglion	0.13	0.61	4.7
	Flight muscle	0.08	0.14	1.8
	Hind-leg muscle	0.06	0.22	3.7
Cockroach	Cerebral ganglion	0.17	0.84	4.9
	Flight muscle	0.08	—	—
Rosechafer	Cerebral ganglion	0.16	—	—
	Flight muscle	0.07	—	—
Honey-bee	Flight muscle	0.03	0.16	5.3
Bumble-bee	Flight muscle	0.24	0.65	2.7
Blowfly	Flight muscle	0.19	—	—
Trout	Liver	<0.07	0.45	>6.4
	Heart	0.05	0.22	4.4
	Red abdominal muscle	0.65	—	—
	White abdominal muscle	<0.52	1.9	>3.7
Frog	Brain	0.61	1.1	1.8
	Liver	0.26	1.50	5.8
	Heart	0.05	0.10	2.0
	Gastrocnemius muscle	0.21	0.40	1.9
Pigeon	Brain	1.5	2.8	1.9
	Liver	0.08	0.37	4.6
	Heart	0.09	0.27	3.0
	Pectoral muscle	0.09	0.46	5.1
Domestic fowl	Brain	2.0	3.8	1.9
	Liver	0.28	1.1	4.0
	Heart	0.13	0.27	2.1
	Pectoral muscle	0.11	0.22	2.0
	Sartorius muscle	0.03	0.11	3.7
Mouse	Brain	1.7	2.9	1.7
	Liver	0.15	0.54	3.6
	Heart	0.45	1.6	3.6
	Gastrocnemius muscle	0.16	0.46	2.9
Rat	Brain	2.2	3.7	1.7
	Liver	0.28	2.1	7.4
	Heart	0.74	3.0	4.1
	Gastrocnemius muscle	0.09	0.30	3.3

stimulated concentrations from 0.10 to 3.8 μM). The measured basal and hormone-stimulated concentrations of cyclic AMP that have been reported for some tissues are presented in Table 9. The ratio basal concentration/stimulated concentration for hormonal effects ranges from 1.7 to 76, but if the effect of glucagon on the liver is excluded (see above) the ratio ranges from 1.4 to 8.6. Thus the calculated variations in the ratio are very similar to those that are measured (see also Table 9). To aid comparison of the measured concentrations of cyclic AMP with those calculated from the model (Table 8), some of the latter values are included in Table 9. Such a comparison is of limited value in providing evidence for the model, since measurements of cyclic AMP concentrations have been restricted primarily to mammalian tissues. Further, the concentrations under similar conditions reported by different authors vary considerably (see Table 9). Nevertheless, in some tissues the predicted and reported concentrations are very similar. It is considered that the available data on cyclic AMP concentrations and changes in these concentrations are in reasonable agreement with those calculated from the model.

Table 9. *Reported and calculated contents of cyclic AMP in different tissues*

The measured contents of cyclic AMP have been taken from the literature but excluding any experiments in which phosphodiesterase inhibitors were used. Where necessary, data reported as μmol/mg of protein have been converted into μmol/g of tissue by assuming that protein represents 10 and 20% of the fresh wt. for brain and other tissues respectively. The calculated contents of cyclic AMP are taken from Table 8 and the calculated maximal rate of increase of cyclic AMP content is the difference between fluoride-stimulated and basal adenylate cyclase activities (see Tables 2–4). Calculated contents are derived from activities measured at 30°C. The data from the literature were obtained at 37°C, except for frog heart (22–25°C) and leg muscle (unrecorded) temperatures, and they are obtained from the following references: (*a*) Brooker (1975); (*b*) Posner *et al.* (1965); (*c*) Breckenridge (1964) and Aurbach & Houston (1968); (*d*) Gilman (1972); (*e*) Lyon & Mayer (1969); (*f*) Burkard (1972); (*g*) Robison *et al.* (1967) and Namm *et al.* (1968); (*h*) Mayer *et al.* (1970).

			Content of cyclic AMP (nmol/g of fresh tissue)				Stimulated content / Basal content		Rate of increase of content of cyclic AMP (nmol/min per g of fresh tissue)	
			Basal conditions		Stimulated conditions					
Animal	Tissue preparation	Hormone addition	Measured	Calculated	Measured	Calculated	Measured	Calculated	Measured	Calculated
Frog (*Rana pipiens*)	Heart ventricle strip during diastole (*a*)	Adrenaline (10 μM)	0.55	0.05	0.92	0.10	1.7	2.0	—	—
(*Rana catesbeiana*)	Muscle *in situ* (*b*)	Adrenaline (50 nmol/kg body wt. injected into heart)	0.59	0.21	1.59	0.40	2.7	1.9	0.1–0.6	0.5
Mouse	Whole brain (*c*)	—	1.1–1.3	1.7	—	—	—	—	—	—
	Liver (*d*)	—	0.95	0.15	—	—	—	—	—	—
	Gastrocnemius muscle *in situ* (*e*)	Adrenaline (25 nmol/kg body wt. injected intravenously)	1.95	0.16	4.8	0.46	2.5	2.9	1.0	1.6
Rat	Whole brain *in situ* (*f*)	Adenosine (0.5 μmol injected into cerebral ventricle)	1.9	2.2	5.0	3.7	2.6	1.7	2.3	80.0
	Perfused liver (*g*)	Adrenaline (0.3 μM)	0.5	0.28	2.0	2.1	4.0	7.4	2.0	11.5
		Glucagon (20 nM)	0.5	0.28	38.0	2.1	76.0	7.4	10.0	11.5
	Heart *in situ* (*h*)	Adrenaline (10 nmol/kg body wt. per min)	0.5	0.74	1.8	3.0	3.6	3.0	7.8	13.2
	Gastrocnemius muscle *in situ* (*b*)	Adrenaline (50 nmol/kg body wt. injected into heart)	0.69	0.09	1.82	0.30	2.7	3.3	1.1	1.7

Table 10. *Ratios of activities of adenylate cyclase/low-K_m cyclic AMP phosphodiesterase and high-K_m cyclic AMP phosphodiesterase/low-K_m cyclic AMP phosphodiesterase*

Data are taken from Tables 2–4. In the mammals and birds division of animals below, the ratio of activities low-K_m phosphodiesterase/fluoride-stimulated adenylate cyclase are tabulated in ascending order.

Animal	Tissue	Activity of low-K_m phosphodiesterase / Activity of fluoride-stimulated adenylate cyclase	Activity of high-K_m phosphodiesterase / Activity of low-K_m phosphodiesterase
Mammals and birds			
Domestic pigeon	Brain	0.2	166.0
Rat	Brain	0.2	119.0
Domestic fowl	Brain	0.3	107.0
Mouse	Brain	0.4	58.0
Rat	Heart	0.5	19.0
Rat	Liver	1.1	25.0
Domestic fowl	Liver	1.7	15.0
Mouse	Heart	1.7	11.0
Domestic pigeon	Pectoral muscle	2.3	10.0
Mouse	Liver	2.4	31.0
Domestic pigeon	Liver	2.5	19.0
Mouse	Gastrocnemius muscle	4.0	4.8
Rat	Gastrocnemius muscle	5.8	4.7
Domestic pigeon	Heart	7.0	2.8
Domestic fowl	Sartorius muscle	8.2	1.2
Domestic fowl	Pectoral muscle	9.6	1.5
Domestic fowl	Heart	9.7	2.4
Others			
Trout	White abdominal muscle	0.8	4.2
	Liver	6.2	2.2
	Heart	12.1	1.0
Frog	Liver	0.9	22.0
	Brain	1.1	38.0
	Gastrocnemius muscle	4.3	11.0
	Heart	8.9	20.0
Locust	Cerebral ganglion	2.3	4.3
	Leg muscle	5.9	3.8
	Flight muscle	11.5	1.4

Rate of turnover of cyclic AMP

If the basal activities of adenylate cyclase reported in this work (Tables 2–4) represent the basal activities *in vivo*, they also indicate the minimum rate of turnover of cylic AMP in the cell. The time for the turnover of the tissue content of cyclic AMP is given by the concentration of cyclic AMP divided by the rate of turnover. Comparison of the data for the rat and mouse in Table 9 and Tables 2–4 indicates that the turnover time is about 60, 10, 3 and 0.3s for gastrocnemius muscle, liver, heart and brain respectively. The rate of turnover of cyclic AMP determines the rate at which the nucleotide concentration can change in response to a change in the activity of adenylate cyclase. The reported rates of increase in the concentrations of cyclic AMP in response to hormones in heart, skeletal muscle and liver are similar to those predicted from adenylate cyclase activities (see Table 9). However, rates of increase in the concentration of cyclic AMP in the brain in response to hormones are much lower than adenylate cyclase activities. This could be explained if only a small proportion of brain cells respond to an individual hormone. Indeed, the change in the concentration of cyclic AMP in response to hormones is very large in single cell-type lines derived from brain (see Brooker, 1975).

The high activities of adenylate cyclase and the consequent high rates of turnover of cyclic AMP in brain and heart tissues may be related to the role of the nucleotide in regulating processes which must be modulated rapidly in these tissues. In nervous tissue, changes in cyclic AMP concentrations may control membrane permeability to ions in the modulation of nervous transmission (see Greengard, 1976; Daly, 1973; Rall, 1975; Williams & Rodnight, 1976). In heart, changes in cyclic AMP concentration may be involved in the transmission of excitation from one muscle fibre to another, since cyclic nucleotide concentrations oscillate in phase with the contraction–relaxation cycle of the heart (Brooker, 1973; Wollenberger *et al.*, 1973).

Knowledge of the rate of turnover of cyclic AMP in any tissue permits an estimation of the importance of extrusion of cyclic AMP from the cell as a means of control of the intracellular concentration. The available information suggests that extrusion does not play a significant role in this respect. For example, the maximal (glucagon-stimulated) rate of release of cyclic AMP from the rat liver is 0.6μmol/min per g of fresh liver (Sherline *et al.*, 1972; Kuster *et al.*, 1973; Pilkis *et al.*, 1974) and the present work indicates that the maximal rate of turnover would be 17μmol/min per g at 30°C: in heart muscle the maximal (isoproterenol-stimulated) rate of release is 0.16μmol/min per g (O'Brien & Strange, 1975), whereas the present work indicates a maximal turnover rate of 25μmol/min per g at 30°C.

Roles of high- and low-K_m phosphodiesterases in the control of cyclic nucleotide concentrations

The model system described above provides an explanation for the presence of both high- and low-K_m phosphodiesterases in tissues. To maintain the concentration of cyclic AMP at a steady state despite variations in adenylate cyclase activity, the total phosphodiesterase activity must exceed that of adenylate cyclase. If this were not the case, hormonal stimulation of adenylate cyclase could cause a large accumulation of cyclic AMP, which might not in itself be harmful to the cell, but it would take a long time to revert to a new steady state after stimulation, so that acute reversible hormonal control would be difficult. Thus in tissues in which the fluoride-stimulated activity of adenylate cyclase is greater than that of low-K_m phosphodiesterase (e.g. rat heart, brain tissue of vertebrates) or in which the ratio activity of low-K_m phosphodiesterase/activity of adenylate cyclase is low, the activity of the high-K_m phosphodiesterase is high compared with the low-K_m phosphodiesterase (see Table 10 and Fig. 1).

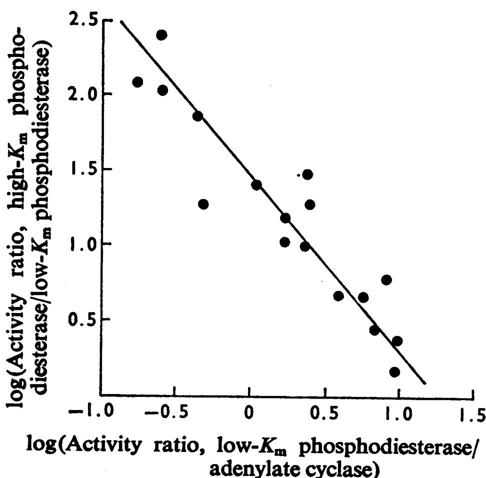

Fig. 1. *Regression plot of the ratios activity of low-K_m phosphodiesterase/activity of adenylate cyclase against log of the ratios activity of high-K_m phosphodiesterase/activity of low-K_m phosphodiesterase*

Only the data for mammals and birds, which are obtained from Table 10, have been used for this plot. The linear correlation coefficient is 0.93 ($P<0.001$).

Table 11. *Theoretical analysis of the effect of different proportions of low- and high-K_m phosphodiesterases on the steady-state concentration of cyclic AMP*

The steady-state concentration of cyclic AMP was calculated for different proportions of low- and high-K_m phosphodiesterases by using the equation

$$V_a = \frac{V_l \cdot s}{K_{ml}+s} + \frac{V_h \cdot s}{K_{mh}+s} \text{ (see the Discussion section)}$$

where $K_{ml} = 1\,\mu$M, $K_{mh} = 20\,\mu$M, $V_a = 10$nmol/min per g and $V_l + V_h = 100$nmol/min per g.

Maximal activities of phosphodiesterase (nmol/min per g)		Steady-state concentration of cyclic AMP
High-K_m enzyme (V_h)	Low-K_m enzyme (V_l)	(μM)
0	100	0.11
25	75	0.15
50	50	0.23
75	25	0.49
100	0	2.22

Although a high-K_m phosphodiesterase alone would be sufficient to provide satisfactory control of the concentration of cyclic AMP, the presence of both high- and low-K_m enzymes has the advantage that a lower total phosphodiesterase protein concentration provides sufficient catalytic activity to maintain the steady-state concentration of cyclic AMP (i.e. the low-K_m enzyme, since it is operating nearer its maximal activity, is a more efficient catalyst than the high-K_m enzyme). However, it is unclear why the ratio of phosphodiesterase activities (high-K_m/low-K_m) should vary between different tissues (see Table 10). Two possible explanations are proposed below. First, variations in the kinetics of the phosphodiesterase system due to different proportions of the high- and low-K_m enzyme could produce variations in the basal concentrations of cyclic AMP in different tissues. If the total activity of phosphodiesterase is maintained at the value necessary to prevent accumulation of cyclic AMP, but the proportion of the high-K_m enzyme is increased, the steady-state concentration of cyclic AMP will be increased (see Table 11). The proportion of the high-K_m enzyme is high in brain tissue of vertebrates (Table 10) and it is known that this tissue contains higher concentrations of cyclic AMP

than do other vertebrate tissues (see Table 9). Secondly, variations in the activities of low- and high-K_m phosphodiesterases between tissues may be due to different emphasis on control mechanisms in various tissues. For example, in some tissues the activity of the low-K_m enzyme may be modified by insulin and the activity of the high-K_m enzyme by cyclic GMP (Sakai *et al.*, 1974; Loten & Sneyd, 1970; Russell *et al.*, 1973; Pilkis *et al.*, 1974; Pawlson *et al.*, 1974; Teresaki & Appleman, 1975). The low activity of the low-K_m enzyme in brain, which is insensitive to insulin, would be consistent with this suggestion.

Extended model for the control of cyclic AMP concentration

A further explanation for the presence of two phosphodiesterase enzymes in tissues is that they are localized in different parts of the cell, so that, near the cell membrane, changes in the concentration of cyclic AMP are more sensitive to changes in the activity of adenylate cyclase. In the simple model, discussed above, no localization of the phosphodiesterases is assumed, so that the concentration of cyclic AMP is evenly distributed throughout the cell and it is approximately proportional to the activity of adenylate cyclase. However, if low-K_m phosphodiesterase is situated at a membrane site near to adenylate cyclase, whereas the high-K_m enzyme is situated in the cytosol (see Appleman *et al.*, 1973; Armstrong *et al.*, 1974; Van Inwegen *et al.*, 1975), the low-K_m enzyme will have access to newly synthesized cyclic AMP before the high-K_m enzyme. Consequently, two processes will compete for cyclic AMP: hydrolysis by low-K_m phosphodiesterase at the cell membrane and diffusion to high-K_m phosphodiesterase in the cytosol (followed by hydrolysis). Thus the activity of the low-K_m phosphodiesterase will increase asymptotically towards a maximum as the concentration of cyclic AMP near the cell membrane increases, but the rate of diffusion will increase linearly. Further, if the maximal activity of adenylate cyclase is higher than the maximal activity of low-K_m phosphodiesterase, changes in the concentration of cyclic AMP will be very sensitive to variations in the activity of adenylate cyclase. A mathematical analysis of a similar model has been presented (Boeynaems *et al.*, 1974; Swillens *et al.*, 1974) to explain the greater increase in concentration of cyclic AMP than that of the activity of adenylate cyclase in horse thyroid gland after stimulation by thyroid-stimulating hormone (Boeynaems *et al.*, 1974).

The modified model may explain why hormones plus inhibitors of phosphodiesterase have greater than additive effects on the concentration of cyclic AMP in some tissues (Robison *et al.*, 1971, p. 41; Kakiuchi & Rall, 1968; Butcher *et al.*, 1968). Thus in the presence of a phosphodiesterase inhibitor the activity of adenylate cyclase is readily increased above that of the low-K_m phosphodiesterase. The separate locations for the low- and high-K_m phosphodiesterases suggested above would cause an uneven distribution of cyclic AMP in the cell. Immunohistochemical studies indicate that cyclic AMP accumulates near the cell membrane when the activity of adenylate cyclase is stimulated (Bloom *et al.*, 1973).

The simple model (which assumes an even distribution of cyclic AMP) may underestimate the steady-state concentration of cyclic AMP, because it does not account for the accumulation of cyclic AMP near the cell membrane (see above). In those cases where there is a discrepancy between the calculated concentration of cyclic AMP and the measured concentration, it is indeed found that the calculated concentration is too low (see Table 9).

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