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Guanylate Cyclase

SUBCELLULAR DISTRIBUTION IN CARDIAC MUSCLE, SKELETAL MUSCLE, CEREBRAL CORTEX AND LIVER

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1. Guanylate cyclase of every fraction studied showed an absolute requirement for Mn^{2+} ions for optimal activity; with Mg^{2+} or Ca^{2+} reaction was barely detectable. Triton X-100 stimulated the particulate enzyme much more than the supernatant enzyme and solubilized the particulate-enzyme activity. 2. Substantial amounts of guanylate cyclase were recovered with the washed particulate fractions of cardiac muscle (63–98%), skeletal muscle (77–93%), cerebral cortex (62–88%) and liver (60–75%) of various species. The supernatants of these tissues contained 7–38% of total activities. In frog heart, the bulk of guanylate cyclase was present in the supernatant fluid. 3. Plasma-membrane fractions contained 26, 21, 22 and 40% respectively of the total homogenate guanylate cyclase activities present in skeletal muscle (rabbit), cardiac muscle (guinea pig), liver (rat) and cerebral cortex (rat). In each case, the specific activity of this enzyme in plasma membranes showed a five- to ten-fold enrichment when compared with homogenate specific activity. 4. These results suggest that guanylate cyclase, like adenylate cyclase, and ouabain-sensitive $Na^+ + K^+$-dependent ATPase (adenosine triphosphatase), is associated with the surface membranes of cardiac muscle, skeletal muscle, liver and cerebral cortex; however, considerable activities are also present in the supernatant fractions of these tissues which contain very little adenylate cyclase or ouabain-sensitive $Na^+ + K^+$-dependent ATPase activities.

Guanylate cyclase (EC 4.6.1.2) has been the subject of intensive study in several laboratories in recent years. Many investigators (Hardman & Sutherland, 1969; White & Aurbach, 1969; Schultz *et al.*, 1969) have shown that the supernatant fractions of various tissues contained the bulk of guanylate cyclase activity. However, Ishikawa *et al.* (1969) observed that most of this enzyme was particulate in rat intestine, whereas Gray *et al.* (1970) reported that the sea-urchin sperm enzyme was entirely particulate. Since then, Kimura & Murad (1974), as well as Chrisman *et al.* (1975) noted that the particulate and soluble guanylate cyclase from rat heart and lung displayed different kinetic properties and have concluded that two forms of guanylate cyclase are present in these tissues. Interestingly, Gray & Drummond (1973) showed that most of the enzyme activity in sea-urchin sperm was associated with the flagellar plasma membranes; Schultz *et al.* (1972) found that a small proportion of rat kidney homogenate activity was present in particulate preparations, presumed to contain plasma membranes. By using the non-ionic detergent Triton X-100, significant guanylate cyclase activities were shown to be associated with the particulate fractions of tissues that had previously been shown to contain negligible activities (Schultz *et al.*, 1972; Kimura & Murad, 1974; Chrisman *et al.*, 1975).

In the present study the subcellular distribution of guanylate cyclase has been investigated in heart, skeletal muscle, brain and liver. Our results demonstrate that significant amounts of this enzyme are associated with the washed particulate fractions as well as the plasma membrane-enriched fractions isolated from these tissues. Preliminary reports of some of these findings have already appeared in abstract form (Sulakhe & St. Louis, 1975; Sulakhe *et al.*, 1975; St. Louis, 1975).

Experimental

Materials

[α-^{32}P]GTP (5.34–20.4 Ci/mmol), [α-^{32}P]ATP (9.23–18.0 Ci/mmol), cyclic [^{3}H]AMP (38.4 Ci/mmol), cyclic [^{3}H]GMP (2.11 Ci/mmol), [U-^{14}C]ATP (417 mCi/mmol) and [8-^{14}C]GTP (48.4 mCi/mmol) were purchased from New England Nuclear Corp., Montreal, Que., Canada. Unlabelled nucleotides, nucleosides, purines and pyrimidines, creatine

phosphate, and creatine phosphokinase were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. and P.L. Biochemicals, Milwaukee, WI, U.S.A. Disposable columns (0.7cm×4cm) were obtained from Bio-Rad Laboratories, Richmond, CA, U.S.A. Neutral alumina (WN-3) was purchased from Sigma. Lead sulphate and inorganic salts were purchased from Fisher Scientific Co., Montreal, Que., Canada. All reagents were either of analytical or of reagent grade. Solutions were prepared, and all glassware and plasticware were thoroughly rinsed, with deionized glass-distilled water.

Methods

All tissue-fractionation procedures were carried out at 4°C.

Isolation of sarcolemma from skeletal muscle and cardiac muscle. Rabbit skeletal-muscle sarcolemma were isolated essentially as described previously by Sulakhe *et al.* (1973*a*): guinea-pig cardiac sarcolemma were isolated by the procedure of P. V. Sulakhe *et al.* (1976).

Isolation of liver plasma membranes. Liver plasma membranes were isolated by zonal centrifugation by a method modified from that described by Smigel & Fleischer (1974). Briefly, about 100g of rat liver was minced, suspended in 4vol. of sucrose (10%, w/w)/Hepes [2-(*N*-2-hydroxyethylpiperazin-*N'*-yl)ethanesulphonic acid] (10mM) buffer, pH7.5, and homogenized in a 40ml Dounce homogenizer (Kontes, Vineland, NJ, U.S.A.) fitted with a loose glass pestle (type A). The homogenate was diluted to 5vol. with sucrose (1%, w/w)/Hepes (10mM) buffer, filtered through six layers of cheese cloth and centrifuged for 30min at $20000g_{av.}$. The resulting pellet was resuspended in sucrose (65%, w/w)/Hepes (10mM)/$MgCl_2$ (1mM) buffer, pH7.5, and adjusted to 51% (w/w) sucrose by adding sucrose (10%, w/w)/Hepes (10mM)/$MgCl_2$ (1mM) buffer. To prepare the 'crude membrane fraction', this suspension was edge-loaded into the zonal rotor containing a linear sucrose gradient ranging from 25 to 50% (w/w). A 52% (w/w) sucrose cushion layer was added to fill the rotor, and the material was centrifuged for 1h at $110000g_{av.}$. The membrane material was collected between sucrose gradients of 30–41% (w/w), diluted in 3vol. of ice-cold water, and centrifuged for 30min at $20000g_{av.}$. The pellets were resuspended in sucrose (65%, w/w)/Hepes (10mM)/$MgCl_2$ (1mM) buffer, pH7.5, and the sucrose concentration was adjusted to 41% (w/w). A 'purified membrane fraction' was then prepared by edge-loading the 'crude membrane fraction' into a zonal rotor containing a linear sucrose gradient (30–40%, w/w) in Hepes (10mM)/EDTA (1mM) buffer, pH7.5. A cushion layer of 42% (w/w) sucrose was added to rotor capacity and the material centrifuged for 1h at $110000g_{av.}$. The plasma membranes were collected from the gradients between 30 and 36% (w/w), diluted in 3vol. of ice-cold water, and centrifuged for 30min at $20000g_{av.}$. The pellets were washed once in 1mM-$KHCO_3$ and suspended in this medium (protein concentration about 4mg/ml). Portions of freshly prepared plasma membranes were used for enzyme assays and the remainder was stored in liquid N_2. Electron microscopy was used periodically to check the morphological purity of the preparations; the biochemical purity was monitored as a routine by using 5'-nucleotidase (Mitchell & Hawthorne, 1965) as the plasma-membrane marker and cytochrome *c* oxidase (Cooperstein & Lazarow, 1951), glucose 6-phosphatase (Hübscher & West, 1965) and acid phosphatase (Linhardt & Walter, 1965) respectively as markers for mitochondrial, endoplasmic-reticulum and lysosomal contamination.

Isolation of washed particles and supernates from heart ventricles, skeletal muscle and cerebral cortex of various species. Rats, hamsters, mice and guinea pigs were decapitated, whereas rabbits were anaesthetized with pentobarbital (35mg/kg). Hearts were quickly removed, washed thoroughly, and ventricles were homogenized with 10vol. (v/w) of homogenizing buffer by using a Polytron PT-10 homogenizer (15, 25 and then 15s bursts, with a 30s waiting period between bursts; setting 5.5). Whole hearts of some other species were also used. Skeletal muscle (hind leg) of several species was homogenized with the homogenizer as described above. Cerebral cortex of several species (except frog) was homogenized in 10vol. (v/w) of buffer with a motor-driven glass/Teflon homogenizer (loose fitting; 300rev./min; 10 passes). Frog whole brain was used. The homogenates were centrifuged at $40000g_{av.}$ for 1h, and the resulting residues washed by suspending in 8vol. (v/w) of homogenizing buffer and centrifuging at $40000g_{av.}$ for 1h. These homogenates, washed particles and combined supernatants were assayed for guanylate cyclase as described below.

Assay of guanylate cyclase. The reaction mixture (0.15ml) consisted of 50mM-Tris/HCl (pH7.5), 2mM-dithiothreitol, 10mM-theophylline, 0.1% bovine serum albumin, 2mM-cyclic GMP, 1% Triton X-100, 50μg of creatine kinase, 20mM-creatine phosphate, variable amounts of $MnCl_2$ and [α-^{32}P]GTP (sp. radioactivity 25–30c.p.m./pmol). The protein concentration in the assay ranged from 80 to 200μg. Boiled fractions (100°C for 30min), of similar protein content, served as controls for non-enzymic synthesis of cyclic [^{32}P]GMP. The reaction was started by the addition of [α-^{32}P]GTP to the reaction mixture (preincubated for 5min at 30°C) and was carried out for 10min at 30°C. The reaction was terminated by acidifying with 20μl of 0.5M-sodium acetate buffer, pH4.0, and then placing the tubes in a boiling-water bath for 3–5min. Buffer (300μl of 50mM-Tris/HCl, pH7.6) was added to the pre-cooled

tubes, the contents vortex-mixed and centrifuged at 3000rev./min in a PR-6 International refrigerated centrifuge (rotor no. 253). Supernatant (300μl) was added to tubes containing 10μl of cyclic [^{3}H]GMP (1500c.p.m.) and 10μl of 0.2M-sodium pyrophosphate. The contents were mixed and transferred to columns (0.7cm×4cm) packed with 2g of a dry mixture containing neutral alumina/$PbSO_4$ (3:1, w/w). The tubes were rinsed with an additional 300μl of 50mM-Tris/HCl, pH7.6, and transferred to the columns. These columns were eluted with 6ml of 50mM-Tris/HCl, pH7.6, directly into scintillation vials. Cyclic GMP was counted for radioactivity by the addition of 15ml of Bray's (1960) solution in a liquid-scintillation spectrometer (Isocap 300A; Nuclear–Chicago Corp., Des Plaines, IL, U.S.A.). Recovery of cyclic [^{3}H]GMP from these columns was found to be at least 85%.

Assay of adenylate cyclase. The reaction mixture (0.15ml) consisted of 40mM-Tris/HCl (pH7.5), 8mM-theophylline, 2mM-cyclic AMP, 5.5mM-KCl, 15mM-$MgSO_4$, 50μg of creatine kinase, 20mM-creatine phosphate and [α-^{32}P]ATP (sp. radioactivity 20–40c.p.m./pmol). The reaction was started by the addition of [α-^{32}P]ATP to the mixture (preincubated at 37°C for 5min) and was carried out for 10min at 37°C. The reaction was stopped by boiling the tubes for 3–5min. All other details are as described above for the guanylate cyclase assay, except that 10μl of cyclic [^{3}H]AMP (1500c.p.m.) was added before the samples were transferred to the alumina/$PbSO_4$ columns. Recovery of cyclic AMP (as cyclic [^{3}H]-AMP) was at least 85%.

Assays for ouabain-sensitive Na^++K^+-dependent ATPase (adenosine triphosphatase) and Mg^{2+}-dependent ATPase. These were performed as previously described (Sulakhe *et al.*, 1973*b*).

Protein determination. Protein was determined by the method of Lowry *et al.* (1951).

Results

Linearity of rate of cyclic GMP synthesis

The rate of cyclic GMP synthesis was found to be linear with respect to time of incubation (up to 30min) and protein concentration (up to 200μg). Sodium azide (10mM) increased the rate of synthesis by 20%; GTPase activities were decreased by 30% with 10mM-sodium azide.

Dependency on bivalent cations

The activity of guanylate cyclase in every subcellular fraction examined in the present study showed a marked dependency on Mn^{2+} ions. As shown in Fig. 1(*a*) Mg^{2+} or Ca^{2+} ions were not as effective (20–30-fold less) as Mn^{2+} in catalysing the enzyme reaction. For this experiment, washed particles of rabbit heart ventricles were used. In the presence of sub-optimal concentrations of Mn^{2+} (0.5mM; Fig. 1*b*), a small increase in activity (up to 20%) was observed with low concentrations

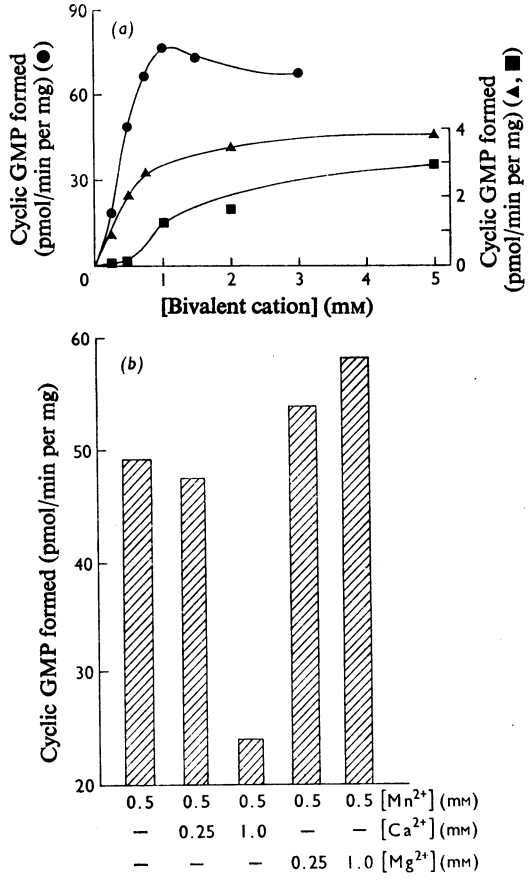

Fig. 1. *Effect of Mn^{2+} (●), Mg^{2+} (■) and Ca^{2+} (▲) on guanylate cyclase*

(*a*) The washed particulate fraction of rabbit heart ventricles was prepared and incubated at 30°C for 10min in the reaction mixture described under 'Methods', except that the bivalent cations were added as shown in this figure. The [α-^{32}P]GTP concentration was 0.75mM and the protein concentration was 163μg/assay. Similar bivalent-cation dependency was observed for guanylate cyclase activity present in the supernatant and other particulate fractions of muscle, liver and cerebral cortex (results not shown). Note that units for guanylase cyclase activity are plotted on two different scales. (*b*) Effect of Mg^{2+} and Ca^{2+} on Mn^{2+}-stimulated guanylate cyclase. Experimental conditions are as described above, and the bivalent-cation concentrations are shown on the abscissa.

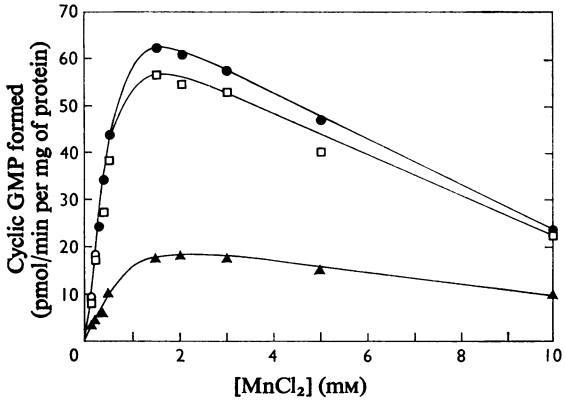

Fig. 2. *Effect of Mn^{2+} on guanylate cyclase*

Rabbit heart ventricular homogenate (10%) and fractions obtained were incubated in the reaction mixture as described in Methods except that [α-^{32}P]GTP was 0.79mM and Mn^{2+} concentrations were as indicated in this Figure. Amounts of protein in the assay were 144μg, 120μg and 139μg for homogenate (□), washed particles (●) and supernatant (▲) respectively. Fractions were incubated for 10min at 30°C.

of Mg^{2+} ions (0.25 and 1.0mM); 1mM-Ca^{2+} inhibited the activity by about 50%. The supernatant-enzyme activity was inhibited 15% at 5mM-Mn^{2+} and 45% at 10mM-Mn^{2+} (compared with maximum activity), whereas the particulate enzyme was inhibited about 30% and 62% at 5 and 10mM-Mn^{2+} respectively (Fig. 2).

Effect of Triton X-100

As a routine, 10–20-fold increases in guanylate cyclase activities of washed particulate and supernatant fractions were observed. In preliminary studies, various types of Lubrols (i.e. P-X, PEX, W etc.) produced effects similar to those of Triton X-100. Hardman *et al.* (1973), Chrisman *et al.* (1975) and Kimura & Murad (1974) have also reported stimulatory effects of Triton X-100. When washed particles were incubated in the presence of Triton X-100, most of the activity was found in the 40000*g*-supernatant fluid (Fig. 3) along with a three- to four-fold purification of the enzyme. During solubilization, high concentrations of Triton X-100 (1%) were slightly inhibitory (Fig. 3). When the particles were exposed for only 5–10min to 1–2% Triton X-100, a similar inhibition was not observed.

Effect of pH and temperature

Both particulate and soluble guanylate cyclase activities were optimal at pH7.5. Increasing the temperature of incubation from 15°C to 40°C increased the activity of particulate enzyme much more than that of the supernatant fraction (Fig. 4). Energies of activation calculated from the slopes of the Arrhenius plot are 13.7 and 7.2kcal/mol for the particulate and soluble enzymes respectively. All determinations were carried out as a routine at 30°C for the studies described in the present paper. Enzyme activities of washed particulate and supernatant fractions were not significantly altered by preincubating these fractions in 0.25M-sucrose/0.2mM-dithiothreitol/10mM-Tris/HCl, pH7.0, at 30°C for up to 40min, especially when the activity was measured in the presence of Triton X-100. Both the particulate and soluble fractions showed a moderate increase (20–30%) in guanylate cyclase activity on preincubation for 10min at 30°C, when assayed in the absence of Triton X-100.

Effect of storage

Guanylate cyclase activities of both soluble and particulate fractions appeared to be fairly stable; there was no demonstrable effect on the activity of this enzyme, when these fractions were kept at room temperature (22–24°C) for 1–2h. Freezing of fractions at −20°C and thawing them at room temperature increased the activity to varying degrees.

Guanylate cyclase in cardiac muscle, skeletal muscle, cerebral cortex and liver of several species

Guanylate cyclase activities in homogenates, washed particles and supernatants of heart ventricles from several species are shown in Table 1. All of these activities were determined in the presence of Triton X-100 in the assay mixture containing optimal Mn^{2+} and GTP concentrations. With the exception of the frog heart, the bulk of the recovered activities (63–98%) was associated with the particulate fractions. The content of guanylate cyclase (expressed as nmol/min per g of tissue at 30°C) was 6.38, 7.30, 9.70, 6.35, 7.87, 0.2, 7.50 in hearts of rat, mouse, guinea pig, hamster, rabbit, frog and clam respectively.

Polytron homogenates prepared from different areas of cardiac tissue (rabbit) contained similar specific activities (40–50pmol/min per mg) of guanylate cyclase. While the present study was in progress, Limbird & Lefkowitz (1975) reported similar findings. Again, the bulk of the recovered activities (75–94%) was present in the washed particulate fractions in every case. The right atrial supernatant contained up to 25% of the total homogenate guanylate cyclase activity (results not shown).

Guanylate cyclase activities in whole homogenates, washed particles and supernatants of skeletal

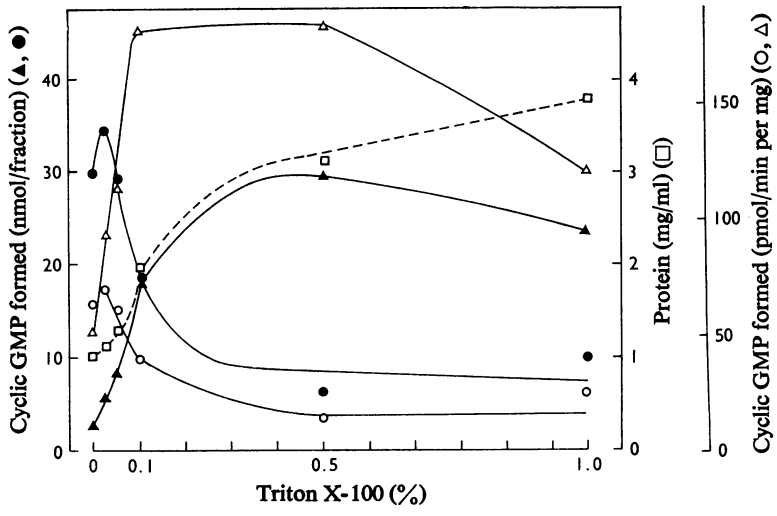

Fig. 3. *Solubilization of particulate guanylate cyclase with Triton X-100*

A washed-particulate fraction of rabbit heart ventricles was isolated as described under 'Methods'. Protein (50mg) was incubated in 5ml of 0.25M-sucrose/0.2mM-dithiothreitol/10mM-Tris/HCl, pH7.0, in the absence and presence of variable amounts of Triton X-100 at 0°C for 30min in centrifuge tubes as shown. The tubes were then centrifuged at 40000$g_{av.}$ for 60min; supernatants were carefully removed, and residues were suspended in 0.25M-sucrose/0.2mM-dithiothreitol/10mM-Tris/HCl, pH7.0. The original washed particles, Triton X-100-insoluble residues and Triton X-100 extracts (supernatant) were assayed for guanylate cyclase activities in reaction mixtures containing 2mM-Mn^{2+}, 1% Triton X-100 and 0.79mM-[α-^{32}P]GTP. □, Protein concentration (mg/ml) of supernatant fluid; ○, specific activity, and ●, total activity, of residue △, specific activity, and ▲, total activity, of supernatant fluid.

muscle and cerebral cortex from different species are shown in Table 2. Enzyme activities (specific activities) in skeletal-muscle homogenates were lowest for frog and highest for guinea pig, and these activities were considerably lower than those observed in cardiac homogenates (Table 1) and cerebral-cortex homogenates (Table 2) of the same species. About 77–93% and 62–88% of the recovered activities were present in the particulate fractions of skeletal muscle and cerebral cortex respectively of different species. The content of guanylate cyclase (nmol/min per g of tissue) of skeletal muscle and of cerebral cortex were 0.67, 1.25, 0.69, 1.58, 0.20, 1.30 and 2.89, 3.22, 4.79, 2.84, 4.3 for rat, hamster, mouse, guinea pig, frog and rabbit respectively. Cerebral-cortex homogenates of frog, guinea pig, and rabbit contained guanylate cyclase of higher specific activity compared with rat, hamster and mouse. Interestingly, the specific activity of the supernatant enzyme of guinea-pig cortex is considerably higher than that of the particulate enzyme.

Guanylate cyclase activities in homogenates of rat and rabbit livers were similar (3–5pmol/min per mg). The content of guanylate cyclase ranged from 0.46 to 0.70nmol/min per mg of liver. About 60–75% of the total guanylate cyclase activity (assayed in the presence of Triton X-100) was present in the washed particulate fractions of rat or rabbit liver (results not shown).

Guanylate cyclase activities of plasma-membrane fractions

As shown in Table 3, plasma membranes of heart and skeletal muscle, liver and cerebral cortex contained significant quantities of guanylate cyclase. Earlier, we had observed that the bulk of adenylate cyclase activity in muscle was present in sarcolemmal membranes (Severson *et al.*, 1972). In the present study, 26% of muscle homogenate guanylate cyclase was found in the isolated sarcolemma, with a purification up to 13-fold. About 21 and 40% of cardiac guanylate and adenylate cyclase activities respectively were present in the plasma-membrane fractions; purification was 6–11-fold. Cardiac plasma membranes contained about 25% of the total ouabain-sensitive Na^++K^+-dependent ATPase activity of homogenate. Liver plasma membranes contained 22 and 48% of guanylate and adenylate cyclase activities respectively; 5′-nucleotidase activities of

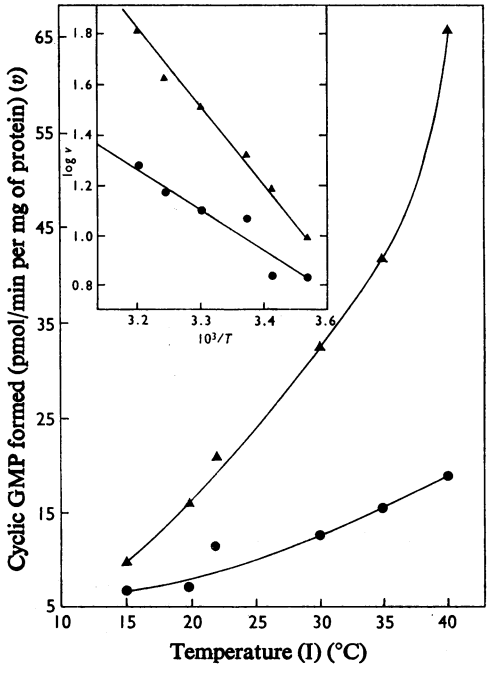

Fig. 4. *Influence of temperature on guanylate cyclase activity*

Washed-particulate (▲) and supernatant (●) fractions were isolated from a 10% (w/v) homogenate of guinea-pig heart ventricles and assayed for guanylate cyclase in the reaction mixtures as described under 'Methods', except that these assays were performed at different temperatures as shown. The inset shows an Arrhenius plot of these data. Amounts of protein in the assays were 90μg and 116μg for washed-particulate and supernatant fractions respectively; [α-^{32}P]GTP, 0.96mM; Mn^{2+}, 2mM; Triton X-100, 1%. Incubations were carried out for 10min.

these membranes showed up to tenfold enrichment, in agreement with previously reported findings (Hickie *et al.*, 1975). The synaptic plasma-membrane fractions, isolated from rat cerebral cortex, according to Jones & Matus (1974), relative to the homogenate, contained 40% ouabain-sensitive Na^++K^+-dependent ATPase, 25% adenylate cyclase (which was also neurohormone-sensitive) and 40% guanylate cyclase activities.

Discussion

In the present study, significant amounts of guanylate cyclase were found in particulate fractions of cardiac and skeletal muscle, liver and cerebral cortex; activities of this enzyme were also detected in the supernatant fractions of these tissues. Our studies also showed that considerable amounts of guanylate cyclase were present in plasma membranes

Table 1. *Distribution of cardiac guanylate cyclase in washed-particulate and soluble fractions*

Fractions were isolated from 10% (w/v) homogenates of heart ventricles of different species as described under 'Methods'. Frozen hearts (−20°C) of clam (*Mercenaria mercenaria*), the fish *Astronotus ocellatus* and parakeet (*Melopsittacus undulapus*) were thawed and homogenized as above. Fractions were incubated in the standard reaction mixtures at 30°C, except that 3mM-Mn^{2+} and 0.96mM-[α-^{32}P]GTP were present. In every case, 85±2% of the total homogenate activity was recovered in these fractions. The number in the parentheses represents the percentage distribution of recovered activity in the isolated fractions.

Species	Guanylate cyclase activity (pmol of cyclic GMP formed/min per mg of protein) of: Homogenate	Washed particles	Supernatant
Rat	43 (100)	48 (91)	13.5 (9)
Hamster	53.5 (100)	55 (86)	26.5 (14)
Mouse	62 (100)	63 (91)	21 (9)
Rabbit	61 (100)	63 (85)	31 (15)
Guinea pig	75 (100)	100 (85)	45 (15)
Fish	49 (100)	78 (91)	15 (9)
Clam	83 (100)	143 (98)	4 (2)
Parakeet	8.9 (100)	6.2 (63)	12.3 (37)
Frog	3.2 (100)	3.1 (18)	15.6 (82)

of the tissues analysed. Kimura & Murad (1974) and Chrisman *et al.* (1975) have reported particulate and soluble guanylate cyclase in rat heart and lung respectively. After the present study was completed, an abstract of Kimura & Murad (1975) and a footnote in a paper by Chrisman *et al.* (1975) both indicated the presence of guanylate cyclase in rat liver plasma membranes.

In agreement with previous reports (Hardman & Sutherland, 1969; White & Aurbach, 1969; Schultz *et al.*, 1969), guanylate cyclase of every fraction required Mn^{2+} for full activity, whereas other cations (Mg^{2+} or Ca^{2+}) were rather ineffective. In the following paper (S. J. Sulakhe, *et al.*, 1976), we show that Ca^{2+} ions can activate guanylate cyclase under certain assay conditions.

The physiological significance of cyclic GMP is not yet known. It has been postulated that cyclic GMP is involved in the actions of cholinergic agents on certain tissues such as cardiac muscle and brain. The evidence that acetylcholine increases cyclic GMP concentration rapidly and selectively in heart ventricles (George *et al.*, 1970; Goldberg *et al.*, 1973), perfused heart (George *et al.*, 1973) as well as cerebral cortex and cerebellum (Kuo *et al.*, 1972), deserves serious consideration but does not by itself, establish any definitive link between muscarinic actions of acetylcholine and cyclic GMP (intracellular). Assuming that the cholinergic (muscarinic)

Table 2. *Distribution and activities of guanylate cyclase in skeletal muscle and cerebral cortex of various species*

Skeletal muscle (leg) of several species was homogenized with a Polytron PT-10 homogenizer in 10vol. of 0.25M-sucrose/10mM-Tris/HCl (pH7.0)/0.2mM-dithiothreitol. Washed particles and supernatants were prepared as described under 'Methods'. Various fractions were incubated for 10min at 30°C as described under 'Methods', except that [α-^{32}P]GTP was 0.8mM. Protein concentrations in the assay ranged from 150 to 180μg, 115 to 160μg and 88 to 100μg for homogenates, washed particles and supernatants respectively. The number in parentheses represents the percentage distribution of the recovered activities (homogenate = 100) in particulate and supernatant fractions of tissue homogenates. The percentage recoveries of guanylate cyclase in fractions were about 85% for all tissues except frog muscle for which the percentage recovery was 190%. Protein recoveries for all tissues were similar (95%). Cerebral cortex of several species (except frog) was homogenized in 10vol. of buffer as described above with a motor-driven glass/Teflon homogenizer (loose fitting, 300rev./min, 10 passes). Frog whole brain was used. Particulate and soluble fractions were isolated and assayed for guanylate cyclase activities as described above. Protein concentrations in the assay ranged from 60 to 120μg, 100 to 125μg and 30 to 60μg for homogenates, washed particles and supernatants respectively. The number in parentheses represents the percentage distribution of the recovered activities (homogenate = 100) in particulate and soluble fractions of homogenates. The percentage recovery of guanylate cyclase was about 80%, whereas that of protein was about 84% for all tissues.

	Guanylate cyclase activity (pmol of cyclic GMP formed/min per mg of protein)					
	Skeletal muscle			Cerebral cortex		
Species	Homogenate	Washed particles	Supernatant	Homogenate	Washed particles	Supernatant
Rat	3.6 (100)	4.4 (78)	3.4 (22)	30.5 (100)	37.5 (88)	12 (12)
Hamster	8.4 (100)	8.8 (91)	2.2 (9)	30 (100)	34 (77)	26 (23)
Mouse	4.4 (100)	5.4 (77)	4.2 (23)	39 (100)	37.5 (77)	27.5 (23)
Guinea pig	11.1 (100)	9.0 (93)	1.8 (7)	56 (100)	43 (62)	70 (38)
Rabbit	8.0 (100)	11.4 (82)	2.0 (18)	97 (100)	120 (75)	102 (25)
Frog	1.3 (100)	3.5 (77)	1.9 (23)	67.5 (100)	44 (87)	19 (13)

Table 3. *Adenylate cyclase and guanylate cyclase, Na^++K^+-dependent ATPase and 5'-nucleotidase activities of isolated plasma-membrane fractions*

Plasma membranes from rabbit leg muscle, guinea-pig heart ventricles, rat liver and rat cerebral cortex were isolated and assayed for enzyme activities as described under 'Methods'. For adenylate cyclase, 12mM-$MgSO_4$, 8mM-NaF and 0.6mM-[α-^{32}P]ATP and, for guanylate cyclase, 1% Triton X-100, 2mM-$MnCl_2$ and 0.6mM-[α-^{32}P]GTP were present in the assay mixtures. The number in parentheses represents the percentage yield of activity.

Tissue	Fraction	Adenylate cyclase		Guanylate cyclase		Ouabain-sensitive Na^++K^+-dependent ATPase	
		(pmol/min per mg)	(%)	(pmol/min per mg)	(%)	(μmol of P_i/h per mg)	(%)
Skeletal muscle (rabbit)	Homogenate	60	(100)	4.0	(100)	0.35	(100)
	Plasma membrane	900	(30)	52.7	(26)	6.0	(32)
Cardiac muscle (guinea pig)	Homogenate	230	(100)	44.0	(100)	1.3	(100)
	Plasma membrane	2510	(40)	260.0	(21)	8.0	(24)
Cerebral cortex (rat)	Homogenate	180	(100)	24.90	(100)	15.0	(100)
	Plasma membrane	720	(25)	145.0	(40)	85.0	(40)
						5'-Nucleotidase	
						(μmol of P_i/h per mg)	(%)
Liver (rat)	Homogenate	10	(100)	15	(100)	3.78	(100)
	Plasma membrane	685	(48)	80	(22)	39.96	(36)

receptor–guanylate cyclase system is operative in some tissues, it would be worthwhile to show experimentally that muscarinic receptors and guanylate cyclase are present in plasma membranes and that muscarinic agonists influence the enzyme activity. Muscarinic receptors have been reported in the particulate fractions of cardiac muscle and cerebral cortex (Bartfai *et al.*, 1974; Yamamura & Snyder, 1974*a*,*b*). In the present study, we have shown that guanylate cyclase is present in plasma-membrane fractions of these tissues. We also found that carbamoylcholine and acetylcholine increased cardiac guanylate cyclase activity, although only moderately (St. Louis, 1975).

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References

Bartfai, T., Ammer, J., Schultchzberg, M. & Montelius, J. (1974) *Biophys. Res. Commun.* **59**, 725–733

Bray, G. A. (1960) *Anal. Biochem.* **1**, 279–285

Chrisman, T. D., Garbers, D. E., Parks, M. A. & Hardman, J. G. (1975) *J. Biol. Chem.* **250**, 374–381

Cooperstein, S. J. & Lazarow, A. (1951) *J. Biol. Chem.* **189**, 665–670

George, W. J., Polson, J. B., O'Toole, A. G. & Goldberg, N. D. (1970) *Proc. Natl. Acad. Sci. U.S.A.* **66**, 398–403

George, W. J., Wilkerson, R. D. & Kadowitz, P. J. (1973) *J. Pharmacol. Exp. Ther.* **184**, 228–235

Goldberg, N. D., O'Dea, R. F. & Haddox, M. K. (1973) *Adv. Cyclic Nucleotide Res.* **3**, 155–233

Gray, J. P. & Drummond, G. I. (1973) *Proc. Can. Fed. Biol. Soc.* **16**, 79

Gray, J. P., Hardman, J. G., Bibring, T. & Sutherland, E. W. (1970) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **29**, 608

Hardman, J. G. & Sutherland, E. W. (1969) *J. Biol. Chem.* **244**, 6353–6370

Hardman, J. G., Chrisman, T. D., Gray, J. P., Suddath, J. L. & Sutherland, E. W. (1973) *Proc. Int. Congr. Pharmacol. 5th*, pp. 134–145

Hickie, R. A., Jan, S.-H. & Datta, A. (1975) *Cancer Res.* **35**, 596–600

Hübscher, G. & West, G. R. (1965) *Nature (London)* **205**, 799–800

Ishikawa, E., Ishikawa, S., Davis, J. W. & Sutherland, E. W. (1969) *J. Biol. Chem.* **244**, 6371–6376

Jones, D. H. & Matus, A. I. (1974) *Biochim. Biophys. Acta* **356**, 276–278

Kimura, H. & Murad, F. (1974) *J. Biol. Chem.* **249**, 6910–6916

Kimura, H. & Murad, F. (1975) *J. Biol. Chem.* **250**, 4810–4817

Kuo, J. F., Lee, T. P., Reyes, P. L., Walton, K. G., Donnelly, T. E. & Greengard, P. (1972) *J. Biol. Chem.* **247**, 16–22

Limbird, L. E. & Lefkowitz, R. J. (1975) *Biochim. Biophys. Acta* **377**, 186–196

Linhardt, K. & Walter, K. (1965) in *Methods of Enzymatic Analysis* (Bergmayer, H., ed.), pp. 779–785, Academic Press, New York

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Mitchell, R. H. & Hawthorne, J. N. (1965) *Biochem. Biophys. Res. Commun.* **21**, 333–338

Schultz, G., Böhme, E. & Munske, K. (1969) *Life Sci.* **8**, 1323–1332

Schultz, G., Jakobs, K. H., Böhme, E. & Schultz, K. (1972) *Eur. J. Biochem.* **24**, 520–529

Severson, D. L., Drummond, G. I. & Sulakhe, P. V. (1972) *J. Biol. Chem.* **247**, 2949–2958

Smigel, M. & Fleischer, S. (1974) *Biochim. Biophys. Acta* **323**, 358–373

St. Louis, P. J. (1975) *Proc. Can. Fed. Biol. Soc.* **18**, 153

Sulakhe, P. V., Drummond, G. I. & Ng, D. C. (1973*a*) *J. Biol. Chem.* **248**, 4150–4157

Sulakhe, P. V., Drummond, G. I. & Ng, D. C. (1973*b*) *J. Biol. Chem.* **248**, 4158–4163

Sulakhe, P. V., Drummond, G. I. & Ng, D. C. (1973*b*) *J. Biol. Chem.* **248**, 4158–4163

Sulakhe, P. V., Leung, N. L. & St. Louis, P. J. (1976) *Can. J. Biochem.* **54**, 438–445

Sulakhe, S. J. & St. Louis, P. J. (1975) *Can. Physiol.* **6**, 57

Sulakhe, S. J., Leung, N. & Hickie, R. A. (1975) *Proc. Can. Fed. Biol. Soc.* **18**, 23

Sulakhe, S. J., Leung, N. L. & Sulakhe, P. V. (1976) *Biochem. J.* **157**, 713–719

White, A. A. & Aurbach, G. D. (1969) *Biochim. Biophys. Acta* **191**, 686–697

Yamamura, H. I. & Snyder, S. H. (1974*a*) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 1725–1729

Yamamura, H. I. & Snyder, S. H. (1974*b*) *Mol. Pharmacol.* **10**, 861–867