Adenylate Cyclase, Guanylate Cyclase and Cyclic Nucleotide Phosphodiesterases of Guinea-Pig Cardiac Sarcolemma

By PATRICK J. ST. LOUIS and PRAKASH V. SULAKHE

*Department of Physiology, College of Medicine, University of Saskatchewan,
Saskatoon, Canada S7N 0W0*

(Received 12 April 1976)

1. The activities of the enzymes involved in the metabolism of cyclic nucleotides were studied in sarcolemma prepared from guinea-pig heart ventricle; the enzyme activities reported here were linear under the assay conditions. 2. Adenylate cyclase was maximally activated by 3 mM-NaF; NaF increased the K_m for ATP (from 0.042 to 0.19 mM) but decreased the K_a for Mg^{2+} (from 2.33 to 0.9 mM). In the presence of saturating Mg^{2+} (15 mM), Mn^{2+} enhanced adenylate cyclase, whereas Co^{2+} was inhibitory. β -Adrenergic amines (10–50 μ M) stimulated adenylate cyclase ($38 \pm 2\%$). When added to the assay mixture, guanyl nucleotides (GTP and its analogue, guanylyl imidophosphate) stimulated basal enzyme activity and enhanced the stimulation by isoproterenol. By contrast, pre-incubation of sarcolemma with guanylyl imidodiphosphate stimulated the formation of an 'activated' form of the enzyme, which did not reveal increased hormonal sensitivity. 3. The guanylate cyclase present in the membranes as well as in the Triton X-100-solubilized extract of membranes exhibited a K_a for Mn^{2+} of 0.3 mM; Mn^{2+} in excess of GTP was required for maximal activity. Solubilized guanylate cyclase was activated by Mg^{2+} only in the presence of low Mn^{2+} concentrations; Ca^{2+} was inhibitory both in the absence and presence of low Mn^{2+} . Acetylcholine as well as carbamoylcholine stimulated membrane-bound guanylate cyclase. 4. Cyclic nucleotide phosphodiesterase activities of sarcolemma exhibited both high- and low- K_m forms with cyclic AMP and with cyclic GMP as substrate. Ca^{2+} ions increased the V_{max} of the cyclic GMP-dependent enzyme.

We described previously a method for the isolation of cardiac sarcolemma in a high degree of purity (P. V. Sulakhe *et al.*, 1976a). The sarcolemmal preparation (fraction F_{3c}) contained significant amounts of adenylate cyclase (EC 4.6.1.1), guanylate cyclase (EC 4.6.1.2) and cyclic nucleotide phosphodiesterase (EC 3.1.4.17) activities. Studies of these enzymes in a highly purified heart sarcolemma have not been reported in the literature. The present observations show some interesting differences in the properties of sarcolemmal adenylate cyclase as well as of cyclic nucleotide phosphodiesterases when compared with those reported for these enzymes present in either washed particulate fractions or soluble fractions from cardiac tissue (Beavo *et al.*, 1970; Drummond *et al.*, 1971; Thompson & Appleman, 1971; Sulakhe & Dhalla, 1973; Tada *et al.*, 1975; Kakiuchi *et al.*, 1975; Sulakhe, 1975). Additionally, cardiac plasma-membrane guanylate cyclase showed stimulation by cholinergic agents.

Methods

Isolation of cardiac sarcolemma

Sarcolemmal fractions were isolated from guinea-

pig heart as described previously (Sulakhe, P. V. *et al.*, 1976a). Sarcolemmal preparations were observed to contain negligible activities of putative marker enzymes for mitochondria, lysosomes and the microsomal fraction (P. V. Sulakhe *et al.*, 1976a); succinate dehydrogenase (EC 1.3.99.1) and cytochrome *c* oxidase (EC 1.9.3.1) were used as mitochondrial markers, acid phosphatase (EC 3.1.3.2) as a lysosomal marker and rotenone-insensitive NADPH-cytochrome *c* oxidoreductase (EC 1.6.2.4) as a microsomal marker. By contrast, adenylate cyclase, guanylate cyclase and ouabain-sensitive ($Na^+ + K^+$)ATPase [($Na^+ + K^+$)-dependent adenosine triphosphatase, EC 3.6.1.3] were enriched five- to ten-fold, compared with homogenate, in the sarcolemmal membranes (St. Louis, 1975; Sulakhe, P. V., *et al.*, 1976a). When examined by electron microscopy, sarcolemmal preparations appeared as empty vesicles of various sizes; occasionally mitochondrial contaminants and some partially extracted fibres were also evident. Sarcolemmal preparations were used within 1 h after isolation for determination of the various enzyme activities. Under the assay conditions used, reaction velocities were proportional to time.

Enzyme assays

Adenylate and guanylate cyclase activities were determined as previously described (P. V. Sulakhe *et al.*, 1976b). Cyclic nucleotide phosphodiesterases were determined by the procedure described by Thompson & Appleman (1971). The assay system (final vol. 0.4 ml) contained 20–75 μ g of enzyme protein, 40 mM-Tris/HCl (pH 8.0), 5 mM-EGTA [ethane-dioxybis(ethylamine)tetra-acetic acid], 3.75 mM-mercaptoethanol, 5 mM-MgCl₂ and various concentrations of [³H]-labelled cyclic nucleotides (48–66 c.p.m./pmol). Snake venom (*Ophiophagus hanna*) was used as the source of nucleotidase for the secondary reaction. Appropriate controls using heat-treated (exposure at 100°C for 30 min) fractions, devoid of phosphodiesterase activity were included. Radioactivity was counted in an Isocap 300A (Nuclear-Chicago) liquid-scintillation spectrometer; the counting efficiency for ³H was about 35%. Protein was determined by the method of Lowry *et al.* (1951).

Results and Discussion

Adenylate cyclase

Effect of NaF. When adenylate cyclase activity was determined with various concentrations of ATP in the absence and presence of NaF, a change in the affinity for ATP, due to NaF, was observed (K_m increased from 0.042 to 0.19 mM). The observed change in the affinity due to NaF is a novel finding for the cardiac enzyme, since earlier work failed to reveal such an effect of NaF (Drummond *et al.*, 1971; Sulakhe & Dhalla, 1973; McNamara *et al.*, 1974). However, NaF increased the V_{max} of the sarcolemmal enzyme (Fig. 1) and the extent of this increase depended on the concentration of Mg²⁺ in the assay; at 2 mM-Mg²⁺, NaF increased the velocity from 0.214 to 1.07 nmol of cyclic AMP/min per mg, whereas at 15 mM-Mg²⁺, V_{max} increased from 0.438 to 0.937 nmol/min per mg. The optimal NaF concentration for the sarcolemmal enzyme was found to be 3 mM (in the presence of 15 mM-Mg²⁺ and 0.75 mM-ATP) and, additionally, there was inhibition of the enzyme in the presence of 15 mM-NaF (basal activity, 500 pmol of cyclic AMP/min per mg; with NaF, 450 pmol/min per mg). Previously, inhibition of the cardiac enzyme by NaF (up to almost 40 mM in the assay) was not observed (Drummond & Duncan, 1970; Sulakhe & Dhalla, 1973; Tada *et al.*, 1975). The nature of this inhibitory effect was not examined further in the present study. However, an inhibitory effect of NaF has been documented for the enzyme from brain (Johnson & Sutherland, 1973) and adipose tissue (Harwood *et al.*, 1973).

Effect of Mg²⁺, Mn²⁺ and Co²⁺. The sarcolemmal enzyme can be saturated with Mg²⁺ and increasing

Fig. 1. Titration of sarcolemmal adenylate cyclase with ATP

(a) Sarcolemmal fraction (66 μ g of protein) was incubated in the standard reaction mixture containing 50 mM-Tris/HCl, pH 7.5, 8 mM-theophylline, 2 mM-cyclic AMP, 5.5 mM-KCl, 50 μ g of creatine kinase, 20 mM-creatine phosphate, in a total volume of 0.15 ml. The reaction was started by the addition of [³²P]ATP to the mixture (preincubated at 37°C for 5 min), and was carried out for 10 min at 37°C. Incubations were carried out in the presence of 2 mM- (●), 7.5 mM- (○) and 15 mM-Mg²⁺ (◐) with 8 mM-NaF present (—) or absent (---). The [³²P]ATP (21.4 c.p.m./pmol) concentration was varied as shown. Adenylate cyclase activity (v) is expressed as pmol of cyclic AMP formed/min per mg of protein. (b) shows the reciprocal of the velocity (v) plotted against the reciprocal of the ATP concentration $1/[S]$.

Mg²⁺ increased the V_{max} of the enzyme (185 pmol/min per mg at 2 mM-Mg²⁺, 403 pmol/min per mg at 15 mM-Mg²⁺). As opposed to its effect on K_m (ATP), NaF caused a decrease in the K_a for Mg²⁺ (2.33 mM, F⁻ absent; 0.9 mM, F⁻ present) at 0.15 mM and 0.74 mM-ATP (Fig. 2). Birnbaumer *et al.* (1969) have noted an increase in the affinity for Mg²⁺ (i.e. decreased K_a) due to the action of NaF on the adenylate cyclase from adipose tissue. When NaF was present in the assay, an S-shaped plot was observed. Drummond & Duncan (1970) previously reported that when the NaF

Fig. 2. Titration of sarcolemmal adenylate cyclase with Mg^{2+} in the absence or presence of NaF

Sarcolemma (51 μ g of protein) was incubated in the standard reaction mixture (see Fig. 1) in the presence of [α - 32 P]ATP (27.83 c.p.m./pmol) at 0.148 mM (○, ●) or 0.75 mM (△, ▲). When included (●, ▲), NaF was 8 mM. Mg^{2+} concentrations were varied as shown in the Figure.

concentration was greater than that of Mg^{2+} , myocardial adenylate cyclase was partially inhibited.

It is well documented that either Mn^{2+} or Co^{2+} can satisfy the bivalent-cation requirement of cardiac adenylate cyclase (Drummond *et al.*, 1971; Perkins, 1973). The results shown in Fig. 3 clearly reveal that in the presence of saturating Mg^{2+} concentrations (15 mM), Mn^{2+} ions further increased the activity of the sarcolemmal enzyme; the increase in the activity in the absence of NaF was less dramatic than that observed in the presence of NaF, and was optimal at 1.0 mM- and 3 mM- Mn^{2+} respectively. On the other hand, under these conditions (i.e. 15 mM- Mg^{2+} present) Co^{2+} ions inhibited the enzyme both in the absence and presence of NaF. Further, the extent of adenylate cyclase stimulation by NaF in the presence of Mg^{2+} plus Mn^{2+} was greater than when only saturating amounts of Mg^{2+} were present.

Effect of isoproterenol and guanyl nucleotides. It has been shown that cardiac adenylate cyclase is stimulated by β -adrenergic amines and also that the nature of this stimulation suggests the coupling of the enzyme to a typical β -receptor (Kaumann & Birnbaumer, 1974; Lefkowitz, 1975). Sarcolemmal adenylate cyclase showed modest but consistent stimulation ($38 \pm 2\%$, mean $\pm$ s.e. of five paired experiments) with β -adrenergic amines (10 μ M–50 μ M). A similar degree of stimulation of adenylate cyclase has been reported for canine (McNamara *et al.*, 1974) and guinea-pig (Tada *et al.*, 1975) cardiac sarcolemmal preparations.

The stimulation due to β -adrenergic agonists (isoproterenol, epinephrine and norepinephrine) was further enhanced in the presence of GTP or its phosphohydrolase-resistant analogue, guanylyl imidodiphosphate. In a typical experiment, the stimulation due to isoproterenol (50 μ M) was increased from 25% to 65% and to 100% in the presence of 2 μ M-GTP and -guanylyl imidodiphosphate respectively. This was consistently observed in many experiments. Additionally, the stimulatory effect of guanyl nucleotides was dependent on the concentration of these nucleotides in the assay mixtures; the maximal stimulatory effect was observed at 50 μ M-GTP (twofold) and 100 μ M-guanylyl imidodiphosphate (fivefold).

When sarcolemma were pre-incubated in the presence of various concentrations of guanylyl imidodiphosphate (either in the absence or presence of isoproterenol) and thoroughly washed by centrifugation, a remarkable activation of the enzyme was observed (Fig. 4). Similar findings were noted with GTP (results not shown). The activation of the enzyme was greater in the presence of isoproterenol

Fig. 3. Effects of Mn^{2+} and Co^{2+} on cardiac-sarcolemmal adenylate cyclase activity in the presence of Mg^{2+} with NaF absent or present

Fractions (45 μ g of protein per assay) were incubated in the standard reaction mixture (see Fig. 1) containing various concentrations of Mn^{2+} (○) or Co^{2+} (△) in the absence (----) or presence (—) of NaF (8 mM). Assay tubes contained 15 mM- Mg^{2+} and 0.75 mM- $[\alpha$ - 32 P]ATP (21.94 c.p.m./pmol).

Fig. 4. Preincubation of sarcolemma with isoproterenol and guanylyl imidodiphosphate

Sarcolemma (2mg of protein in 1.5ml of 0.25M-sucrose/10mM-Tris/HCl, pH7.5) was incubated with various concentrations of guanylyl imidodiphosphate for 7min at 30°C in the absence (●) or presence (○) of 10 μ M-isoproterenol. Inset:—sarcolemma was incubated as above with various concentrations of isoproterenol in the absence (▲) or presence (Δ) of 1 μ M-guanylyl imidodiphosphate. After incubation, all samples were immediately chilled on ice, centrifuged for 15min at 8000 $g_{av.}$, the supernatants discarded and the pellets washed by resuspension in 3.0ml of 0.25M-sucrose/10mM-Tris/HCl, pH7.4, and centrifuging (15min at 8000 $g_{av.}$). The final washed pellets were suspended in 1.0ml of buffer and assayed for adenylate cyclase by using standard assay conditions (see Fig. 1). Assay tubes contained 20–25 μ g of enzyme protein, 15mM-Mg²⁺ and 0.8mM-[α -³²P]ATP (18.18c.p.m./pmol).

plus guanylyl imidodiphosphate in the preincubation mixture and was time- and temperature-dependent. When sarcolemma was preincubated at 10°C, the maximal activation was observed between 15 and 20min, whereas at 30°C, it was reached between 5 and 10min in the presence of guanylyl imidodiphosphate and isoproterenol. In the absence of isoproterenol, longer preincubation times were required for the maximal activation, which was considerably less than that reached in the presence of guanylyl imidodiphosphate and isoproterenol. Further, isoproterenol was found to increase the rate

of activation of the enzyme by guanylyl imidodiphosphate (results not shown). It is evident from these data that exposure to guanylyl imidodiphosphate somehow catalyses the transformation of the enzyme to an 'activated' state and that β -adrenergic agonists (such as isoproterenol) influences the speed and the degree of this 'activation' process. However, such 'preactivated' preparations did not exhibit increased sensitivity to isoproterenol when added in the assay. Similar activation of adenylate cyclase present in other membranes has been described (Cuatrecasas *et al.*, 1975; Lefkowitz & Caron, 1975).

Fig. 5. Effects of Mn^{2+} , Mg^{2+} and Ca^{2+} ions on solubilized guanylate cyclase from cardiac sarcolemma

Sarcolemmal fraction (6.2mg) was treated with Triton X-100 (1% final concn.) for 40 min at 4°C; the preparation was centrifuged for 45 min at 40000g_{av.}, and the supernatant fraction used as the source of enzyme (35 µg of protein/assay). Standard incubation mixtures contained 50mM-Tris/HCl, pH 7.5, 2mM-dithiothreitol, 10mM-theophylline, 1mM-cyclic GMP, 0.1% bovine serum albumin, 50 µg of creatine kinase, 20mM-creatine phosphate and 0.62mM- $[\alpha\text{-}^{32}\text{P}]\text{GTP}$ (14.23 c.p.m./pmol) in a total volume of 0.15 ml. Incubations were carried out at 30°C for 10 min. (a) Effects of various concentrations of Mn^{2+} (○), Mg^{2+} (△) and Ca^{2+} (□); (b) effects of various concentrations of Mg^{2+} (△) or Ca^{2+} (□) in the presence of 0.2mM- Mn^{2+} .

Guanylate cyclase

Effect of Mn^{2+} , Mg^{2+} and Ca^{2+} . Sarcolemmal enzyme, which was solubilized by Triton X-100, was remarkably stimulated by Mn^{2+} (Fig. 5a). [We showed previously that Triton X-100 solubilizes membrane-bound guanylate cyclase (P. V. Sulakhe *et al.*, 1976a). Triton X-100, however, is a powerful inhibitor of adenylate cyclase; about 80–90% inhibition was observed in the presence of 1% Triton X-100.] The K_m for Mn^{2+} was about 0.3mM, which is similar to that observed earlier for the particulate or membrane-bound enzyme (Sulakhe, S. J. *et al.*, 1975, 1976). A Mn^{2+} concentration greater than that of GTP was required for maximal activity, and the plot for activity against $[Mn^{2+}]$ was sigmoidal. Mg^{2+} ions were rather poor activators of the enzyme, whereas in the presence of Ca^{2+} the enzyme activity was inhibited (Fig. 5a, inset). However, in the presence of low Mn^{2+} concentrations ($[GTP]$ greater than $[Mn^{2+}]$) the enzyme was activated by Mg^{2+} , whereas Ca^{2+} ions were inhibitory (Fig. 5b). A stimulatory effect of Ca^{2+} on the enzyme, either solubilized or membrane-bound, was not observed under any conditions. We described

previously (S. J. Sulakhe *et al.*, 1976) that the soluble guanylate cyclase from heart, present in the cytosol, was indeed stimulated by Ca^{2+} in the presence of low concentrations of Mn^{2+} . These results therefore support the view that the particulate guanylate cyclase exists as a metallo-enzyme with Mn^{2+} as the bound metal, and that Mg^{2+} increases the catalytic reactivity; further, fluxes of Ca^{2+} across these membranes could modulate the activity of this enzyme.

Effect of cholinergic agents. Fig. 6 shows that sarcolemmal guanylate cyclase was stimulated by both acetylcholine and carbamoylcholine; the maximal stimulation ($30 \pm 5.5\%$, mean $\pm$ s.e. of four paired experiments) was achieved at 1nM-acetylcholine or 100nM-carbamoylcholine. Atropine, a muscarinic blocker, partially decreased the stimulation due to carbamoylcholine. In other studies with $[\text{H}^3]\text{atropine}$, muscarinic receptors were detected in these membranes, which exhibited specificity (bound $[\text{H}^3]\text{atropine}$ displaced by unlabelled atropine, carbamoylcholine and acetylcholine, but not by nicotine, tubocurarine and β -adrenergic agonists) and saturability (binding constant was 2nM) (S. K. Ma &

Fig. 6. Effects of cholinergic agents on sarcolemmal guanylate cyclase

Guanylate cyclase was assayed as described in Fig. 5 (standard incubation mixture), with guinea-pig cardiac sarcolemma (38 μ g of protein/assay), 2 mM-MnCl₂ and 0.57 mM-[α -³²P]GTP (27.8 c.p.m./pmol). Acetylcholine (Δ) and carbamoylcholine ($\circ$) were added to the assay medium as shown. Atropine, when present ($\bullet$) with carbamoylcholine, was 10 μ M.

P. V. Sulakhe, unpublished results). Limbird & Lefkowitz (1975) have failed to observe effects of cholinergic agents *in vitro* on cardiac guanylate cyclase. Our findings agree with the stimulatory effect of cholinergic agonists on the intracellular concentrations of cardiac cyclic GMP (George *et al.*, 1973). We would like to suggest that changes *in situ* in the concentration of this cyclic nucleotide in heart, due to the action of cholinergic agonists, results from the interactions of these agents with a muscarinic receptor-guanylate cyclase system of sarcolemma.

Cyclic nucleotide phosphodiesterase activities. About 5% of the homogenate phosphodiesterase activities (with either cyclic AMP or cyclic GMP as substrate) were recovered in the isolated sarcolemmal membranes. In guinea-pig ventricles, about 20% of the total phosphodiesterase activities are associated with the particulate fractions (B. L. Russell, S. J. Sulakhe & P. V. Sulakhe, unpublished work). Hence about 25% of the particulate enzyme was recovered in the sarcolemma. As shown in Fig. 7(a), this membrane diesterase exhibited S-shape kinetic curves when titrated with either cyclic AMP or cyclic GMP, both in the

Fig. 7. Cyclic nucleotide phosphodiesterase activities of sarcolemma; titration with cyclic AMP or cyclic GMP

(a) Assays were performed as described in the Methods section, with 61 μ g of protein/assay and [³H]cyclic AMP (Δ , Δ) (63.08 c.p.m./pmol) or cyclic [³H]GMP (48.57 c.p.m./pmol) ($\blacksquare$, $\square$). When included (----), Ca²⁺ was present at 5.125 mM (5 mM-EGTA present). Inset: this is an enlargement of the lower left-hand portion of (a); v is the phosphodiesterase activity expressed as nmol of cyclic nucleotide hydrolysed/min per mg of protein. (b) shows the reciprocal of the velocity (v) plotted against the reciprocal of the cyclic nucleotide concentration, $[S]$. $\square$, Cyclic GMP ($-Ca^{2+}$); $\blacksquare$, cyclic GMP ($+Ca^{2+}$); Δ , cyclic AMP ($-Ca^{2+}$); $\blacktriangle$, cyclic AMP ($+Ca^{2+}$).

absence and presence of Ca^{2+} . Significant stimulation of the diesterase activity by Ca^{2+} was only observed with cyclic GMP as substrate both at low (Fig. 7a, inset) and high (Fig. 7a) substrate concentrations. Lineweaver-Burk analysis of these data is shown in Fig. 7(b), which clearly shows the upward-bending of the curves. One possible interpretation of these data supports the concept of multiple binding sites on the enzyme, with interaction occurring between these sites (Koshland, 1970).

With cyclic AMP as substrate, both high- K_m (270 μM) and low- K_m (1.13 μM) forms of diesterase were detected, the respective $V_{\max}$ being 2.5 nmol/min per mg and 0.022 nmol/min per mg; with cyclic GMP, high- K_m , 166 μM ($V_{\max}$, 1.1 nmol/min per mg, $-\text{Ca}^{2+}$; 1.6 nmol/min per mg, $+\text{Ca}^{2+}$) and low- K_m , 11.8 μM ($V_{\max}$, 0.25 nmol/min per mg, $-\text{Ca}^{2+}$; 0.33 nmol/min per mg, $+\text{Ca}^{2+}$) forms were present. The kinetic properties of the sarcolemmal phosphodiesterases are different from those observed for the cytoplasmic enzyme from cardiac muscle (Goren & Rosen, 1972; Appleman & Terasaki, 1975) and from other tissues (Beavo *et al.*, 1970). These membrane-bound phosphodiesterases can be considered to play a critical role in the regulation of the concentrations of cyclic nucleotides in the vicinity of cardiac plasma membranes. Stimulation of the cyclic GMP-dependent activity by Ca^{2+} suggests the possible regulation of this activity during transsarcolemmal Ca^{2+} movements.

Conclusion

Numerous studies have shown the critical involvement of cyclic nucleotides as well as Ca^{2+} ions in the regulation of myocardial metabolism and function [see Rasmussen *et al.* (1975) for a review]. Our studies provide evidence that the enzymes involved in the synthesis, degradation and action of cyclic nucleotides are present in the plasma membranes of cardiac muscle. Further, cyclases displayed the anticipated modulation of their activities by the respective neurohormones (Figs. 4 and 6), whereas phosphodiesterase (cyclic GMP) was activated by Ca^{2+} ions (Fig. 7). From such observations it can be suggested that the primary alterations occurring in these membranes may subserve the physiological effects of cholinergic-adrenergic agents on heart. The presence of an active Ca^{2+} -transport system in these membranes, and its modulation via protein kinase-catalysed phosphorylation (Hui *et al.*, 1975; St. Louis, 1975; P. V. Sulakhe, *et al.*, 1976a) would further support the view that cardiac sarcolemma is intimately involved in regulation of heart metabolism and function.

This work was supported by a grant from the Saskatchewan Heart Foundation. P. J. St. L. is Research Fellow of the Canadian Heart Foundation. We are grateful to Professor George Drummond for sending us his manuscript before publication.

References

- Appleman, M. M. & Terasaki, W. L. (1975) *Adv. Cyclic Nucleotide Res.* **5**, 156-162
- Beavo, J. A., Hardman, J. G. & Sutherland, E. W. (1970) *J. Biol. Chem.* **245**, 5649-5655
- Birnbaumer, L., Pohl, S. L. & Rodbell, M. (1969) *J. Biol. Chem.* **244**, 3468-3478
- Cuatrecasas, P., Jacobs, S. & Bennett, V. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1739-1743
- Drummond, G. I. & Duncan, L. (1970) *J. Biol. Chem.* **245**, 976-983
- Drummond, G. I., Severson, D. L. & Duncan, L. (1971) *J. Biol. Chem.* **246**, 4166-4173
- George, W. J., Wilkerson, R. D. & Kadowitz, P. J. (1973) *J. Pharmacol. Exp. Ther.* **184**, 228-235
- Goren, E. N. & Rosen, O. M. (1972) *Arch. Biochem. Biophys.* **153**, 384-397
- Harwood, J. P., Low, H. & Rodbell, M. (1973) *J. Biol. Chem.* **248**, 6239-6245
- Hui, E. C., Drummond, G. I. & Drummond, M. (1975) *Adv. Cyclic Nucleotide Res.* **5**, 839
- Johnson, R. A. & Sutherland, E. W. (1973) *J. Biol. Chem.* **248**, 5114-5121
- Kakiuchi, S., Yamazaki, R., Tashima, Y., Unishi, K. & Miyamoto, E. (1975) *Biochem. J.* **146**, 109-120
- Kaumann, A. J. & Birnbaumer, L. (1974) *J. Biol. Chem.* **249**, 7874-7885
- Koshland, D. E., Jr. (1970) *Enzymes 3rd Ed.* **1**, 340-396
- Lefkowitz, R. J. (1975) *Biochem. Pharmacol.* **24**, 583-590
- Lefkowitz, R. J. & Caron, M. G. (1975) *J. Biol. Chem.* **250**, 4418-4422
- Limbird, L. E. & Lefkowitz, R. J. (1975) *Biochim. Biophys. Acta* **377**, 186-196
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- McNamara, D. B., Singh, J. N. & Dhalla, N. S. (1974) *J. Biochem.* **76**, 603-609
- Perkins, J. P. (1973) *Adv. Cyclic Nucleotide Res.* **3**, 1-74
- Rasmussen, H., Jensen, P., Lake, W., Friedman, H. & Goodman, D. P. B. (1975) *Adv. Cyclic Nucleotide Res.* **5**, 375-394
- St. Louis, P. J. (1975) *Proc. Can. Fed. Biol. Soc.* **18**, 153
- Sulakhe, P. V. (1975) *Proc. Can. Fed. Biol. Soc.* **18**, 113
- Sulakhe, P. V. & Dhalla, N. S. (1973) *Biochim. Biophys. Acta* **293**, 379-396
- Sulakhe, P. V., Leung, N. L. & St. Louis, P. J. (1976a) *Can. J. Biochem.* **54**, in the press
- Sulakhe, P. V., Sulakhe, S. J., Leung, N. L., St. Louis, P. J. & Hickie, R. A. (1976b) *Biochem. J.* **157**, 705-712
- 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
- Tada, M., Kirchberger, M. A., Iorio, J. M. & Katz, A. M. (1975) *Circ. Res.* **36**, 8-17
- Thompson, W. J. & Appleman, M. M. (1971) *Biochemistry* **10**, 311-316