Inverse Regulation of Calcium Channels and β-adrenergic Receptors in Virus-Transformed Human Embryonal Cells

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Abstract. Monolayer cultures of human embryonal smooth muscle cells (HEC) were used to study the heterologous regulation of membrane β-adrenergic receptors and Ca^{2+} channels. The relationships between the activation of membrane bound alpha-1 and β-adrenergic receptors, the cyclic nucleotide response and Ca^{2+} channel binding were studied in a cellular model of latent virus infection (Herpes simplex, Type-2) in a human embryonal cell line. In the early stage of infection (72 h), there was a significant increase in the cell cAMP content, followed by a decrease in the binding capacity of the β-adrenergic ligand with an increased total number of the 1,4-dihydropyridine Ca^{2+} channel agonist (-)-S-(^{3}H)BAYK 8644 binding sites on the cell membrane of infected cells. The increased numbers of Ca^{2+} agonist binding sites were accompanied by an increased cAMP content in the cells and an increased membrane ATP-ase activity. Down-regulation of (^{3}H)DHA binding, and an increased capacity of Ca^{2+} agonist binding were found after prolonged exposure of HEC to isoprenaline (10^{-5} mol.l^{-1}). Stimulation of alpha-1 adrenergic receptors with phenylephrine (10^{-6} mol.l^{-1}) was accompanied with only slight but significant increase in (^{3}H)DHA binding and with a significant reduction in the total number of Ca^{2+} channel agonist binding sites.

Key words: Calcium channels — Human embryonal cells — β-adrenergic receptors — Ligand binding — Virus-transformed cells — (-)-S-(^{3}H)BAYK 8644 — (^{3}H)DHA

Introduction

Some viruses penetrate the host cell membrane, slip into the cell genome and reside there indefinitely rather than killing the cell (Hirsch 1988). As result of membrane defects, the infected cells are mostly in an anomalous functional

state, with disturbances of specialized cellular functions (Oldstone 1989). Smooth muscle permeabilized with pore-forming protein have recently been found to have extremely valuable properties of retaining receptors coupled to a major effector system (Ahnert-Hilger and Grabel 1988). The viruses, rather than poration, attach to cells as a result of binding of a viral attachment protein to a site on a host-cell receptor. This type of interaction of virus -protein with the cell membrane is followed by the entry of virus into the cell either by direct fusion with the plasma membrane or by receptor-mediated endocytosis (Paulson 1985). Similarities of viral protein with normal protein ligands for the host cell receptors, in addition to allowing the virus to enter the cell, may also be responsible for certain biological effects of viruses that are unrelated to infection. Any normal constituent of the cell membrane represents a potential virus receptor. These include phospholipids, integral membrane proteins and possibly also enzymes, ion channels, receptors and transporters for neurotransmitters and hormones (Lentz 1988).

As we recently reported, cultured human embryonal smooth muscle cells are a rich source of both, the β-adrenergic and the Ca^{2+} agonist binding sites (Dřímal 1989). Furthermore, down-regulation of β-adrenergic binding sites induced by exposure to isoproterenol was accompanied by an increased binding of a Ca^{2+} channel agonist (Dřímal 1989). In vitro viral exposure was reported to diminish β-adrenergic response in human leucocytes (Lee 1980).

In the present study, the binding properties of the calcium activating 1,4-dihydropyridine (-)-S-(^{3}H)BAYK 8644 and the β-adrenergic receptor ligand (^{3}H)DHA, as well as the functional β-adrenergic response were studied in monolayer cultures of human embryonal smooth muscle cells (HEC) after controled manipulation of the cell interior during early stages of virus infection.

Materials and Methods

Chemicals: (-)-S-(5methyl(^{3}H)BAYK 8644, specific activity 71.7 Ci mmol (NEN Res. Product, DuPont, Boston Ma, USA); (^{3}H)dihydroalprenolol (DHA), specific activity 60 Ci mmol (Izinta Hungary); nitrendipine (Bayer AG, FRG); izoprenaline-hydrochloride (Fluka); propranolol (ICI); BAYK 8644 (Calbiochem); l-phenylephrine-hydrochloride (Sigma); Tris(hydroxymethyl)aminomethane (Serva). Serum for cell cultures and other cell culture reagents were from Gibco (Grand Island, N.Y., USA).

Cell Culture: Human embryonal cells (HEC) were generously donated by Dr. J. Závada and Dr. G. Russ (Institute of Virology, Slovak Academy of Sciences). The cell type used was characterized elsewhere (Závada et al. 1983).

HEC were cultured at 37 °C in an atmosphere of 10% CO_2 in a complete medium consisting of 90% Dulbeco's modification of Eagle's minimal essential medium, glucose and 10% fetal bovine serum. Cell monolayers were washed twice with the cultivation medium without glucose and serum

and then incubated for 30 min with media containing tritiated ligands and competing compounds. All experiments were performed with cells derived from the third to sixth subculture. Confluent cells (mostly day 4 in culture) had spindle-like morphology, and produced characteristic "hills and valleys" growth.

Infected Cells: Cells were infected with Herpes simplex virus type-2 (HSV-2) as described by Závada et al. (1983). Infected cells were harvested 72 hrs after infection. The titre of HSV assayed were 3.7×10^{-7}/ml plaque forming units.

Binding Assays: (-)-S-(^{3}H)BAYK 8644 and (^{3}H)DHA binding to the whole cell preparations were measured as described by Toews (1986) and Dřimal (1989). At the end of 1 h and 18 h incubation period both, control and infected cells were scraped off of the plates and harvested onto Wathman GF/C glass fiber filters. The cells were washed twice with 3.0 ml assay buffer, and radioactivity bound on the filters was collected in 10 ml of scintillation cocktail (Spolana, Neratovice, Czecho-Slovakia) and counted in the Rack β-Liquid Counter (LKB Wallace, Turku, Finnland) at an efficiency of 65%.

Cyclic-AMP Assays: The cyclic-AMP assays were performed as described by Dřimal et al. (1987) except that cell monolayers were washed with buffer, the cells were homogenized with an Ultra-Turrax homogenizer, the homogenate was centrifuged, and the cAMP content was determined by the (^{125}I)RIA method, (IRAPRA, Prague).

Mg^{2+} ATP-ase: Mg^{2+}ATP-ase activity was measured according to Pullmann (1967): The assay mixture (pH 7.51) contained (in mmol.l^{-1}): sucrose 0.25; Tris -acetate 50.0; EDTA 0.5; glucose 25.0; $MgCl_2$ 5.0; potassium phosphate 20.0; and 1.5×10^4 cpm ^{32}P (specific activity 5 mCi/g) and hexokinase (Typex 10—30 μ/mg protein) 70 μg. Protein was assayed by method of Bradford (1976).

Statistics: Data reported in this study are mean values $\pm$ SEM. Student's t-test for either paired or unpaired observations was used at the $P < 0.05$ level.

Results

The confluent human embryonal smooth muscle cells, in addition to the characteristic "hills and valley growth" (not shown) responded to homologous serum in a typical manner. The cells grown in the medium containing human serum exhibited a distinctive growth. Control or infected cells incubated in the medium containing pharmacologically active substances (im μmol.l^{-1}), phenylephrine (1.0), trifluperazine (10.0), nonachlazine (20.0), nitrendipine (1.0) or propranolol (10.0) showed no evidence of lysis, when compared by light microscopy with control cells. Mortality (Trypan blue 0.5 %) in the control group and in the phenylephrine, phenothiazine and nitrendipine group was $3.8 \pm 1.2\%$. A relatively higher mortality ($6.4 \pm 2.4\%$) was observed after incubation with isoprenaline (18 h) in control and also in infected HEC.

Previously we could demonstrate in HEC the presence of β-adrenergic binding sites with characteristics of a beta-adrenergic receptor (Dřimal 1989). The

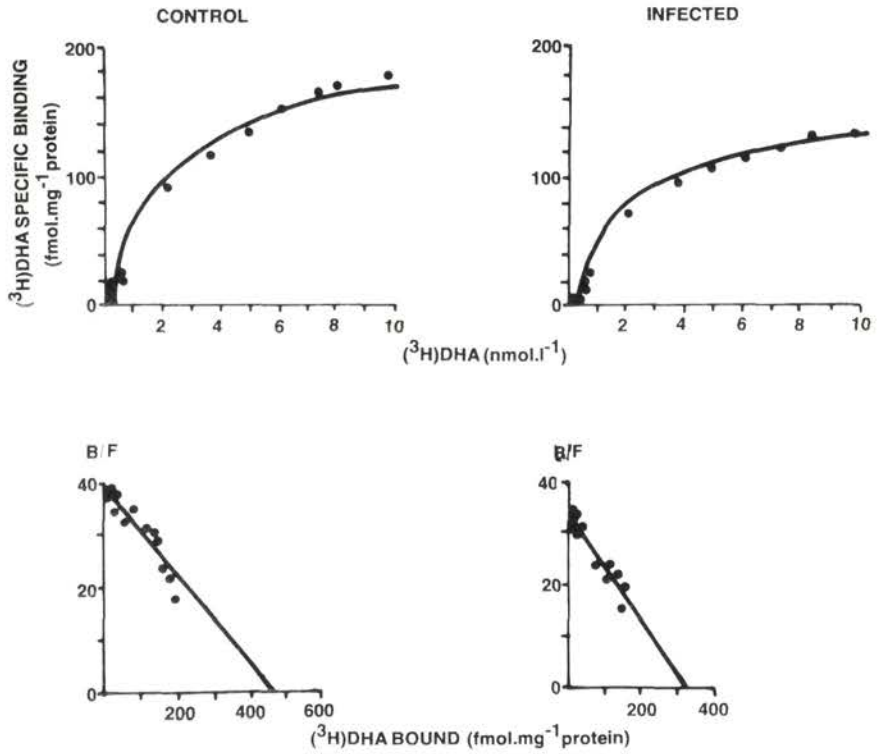

Fig. 1. *A*: Specific (^{3}H)DHA binding in cultured human embryonal smooth muscle cells as a function of the maximal concentration of the ligand. Nonspecific bindig was determined in the presence of 50 μmol.l^{-1} dihydroalprenolol in triplicate assays; each point represents the mean of 12 determinations. *B*: Scatchard plot for specific binding of (^{3}H)DHA. Ordinate: Bound over free (B/F) (^{3}H)DHA; Abscissa: (^{3}H)DHA in fmol.mg^{-1} protein

results of saturation binding studies with (^{3}H)DHA are shown in Fig. 1. In the present study, the numbers of binding sites found in infected cells were significantly reduced ($-30.8 \pm 5.0\%$, $P < 0.01$), without any significant alteration in affinity ($K_d = +12.1 \pm 19\%$). The reduction in B_{max} values for (^{3}H)DHA was neither explained by changes in HEC numbers nor by those in the protein content of the infected cells (control 0.950 ± 0.30 mg/g cell; infected $0.980 \pm 0{,}31$ mg/g cell). There was only a small and nonsignificant increase in the mortality in this group of experiments ($3.9 \pm 1.6\%$). The specific binding of the 1,4-dihydropyridine Ca^{2+} agonist (-)-S-(^{3}H)BAYK 8644 (0.14—3.0 nmol.l^{-1}) on HEC was saturable, forming a plateau in the 0.5—3.0 nmol.l^{-1} region

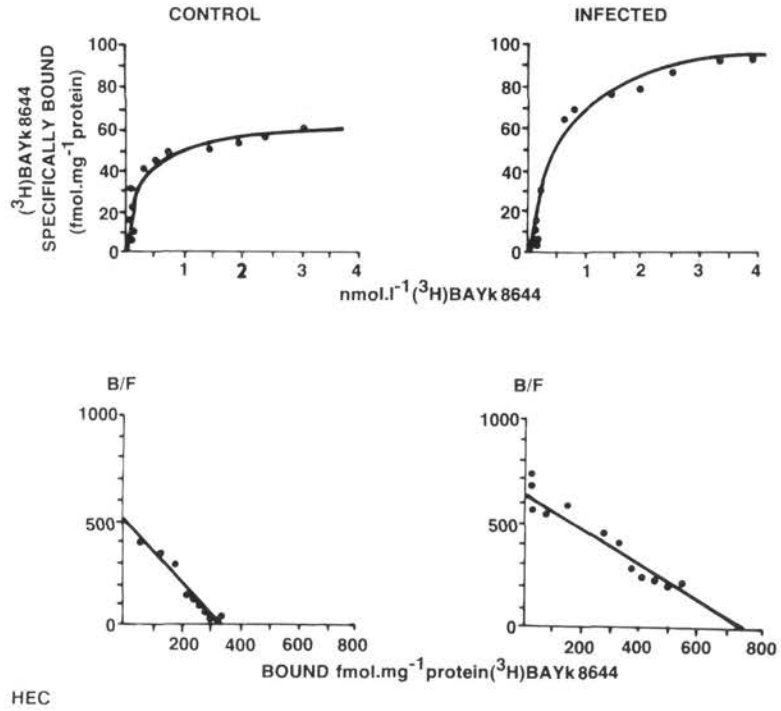

Fig. 2. Specific binding of (-)-S-(^{3}H)BAYK 8644 in whole cell human embryonal smooth muscle cell preparations. Specific binding was determined as the difference between total and nonspecific binding in the presence of 1.0 μmol.l^{-1} BAYK 8644. B_{max} for controls = 252 ± 11 fmol.mg^{-1} protein; K_d = 0.69 ± 0.29

(Fig. 2). The B_{max} value for (-)-S-(^{3}H)BAYK 8644 in this group of experiments was 448 ± 11.0 fmol.mg^{-1} protein, and it showed a high affinity (K_d = 0.69 ± 0.29 nmol.l^{-1}). The number of binding sites in infected cells increased markedly (+167 ± 12%, $P < 0.01$).

Effects of drugs on specific binding: A number of drugs, mostly activators or specific antagonists of clinically relevant categories) were tested for their ability to affect the calcium channel agonist binding to HEC. The β-adrenergic receptor agonist isoprenaline (Table 1) caused a significant reduction in the total number of (^{3}H)DHA binding sites in HEC. The reduction in the (^{3}H)DHA binding went mostly on the account of a reduced affinity (+306 ± 9% increase in the numeric values of K_d). Specific binding of (-)-S-(^{3}H)BAYK 8644 in this group of experi-

Table 1. 1,4-Dihydropyridine Ca^{2+} agonist and β-adrenergic ligand binding sites in human embryonal cells

PROCEDURE	LIGAND (B_{max} fmol . mg^{-1} protein) (K_d nmol . l^{-1})		
(*a*)	(-)-S-(^{3}H)BAYK 8644		(^{3}H)DHA
CONTROL	B_{max} K_d	448.0 ± 11.0 0,69 ± 0.06	2.470 ± 42,0 5.80 ± 0.80
(*b*)	(-)-S-(^{3}H)BAYK 8644		(^{3}H)DHA
INFECTED (n = 12)	B_{max} K_d	+167.0 ± 12* +152.1 ± 68*	−30.8 ± 5* +12.1 ± 19
ISOPRENALINE (n = 10)	B_{max} K_d	+184.0 ± 21* +26.2 ± 13	−79.6 ± 10* +306.2 ± 9*
PHENYLEPHRINE (n = 12)	B_{max} K_d	−57.1 ± 10* −21.2 ± 11*	+21.7 ± 13 +54.5 ± 23
TRIFLUPERAZINE + Isoprenaline (n = 8)	B_{max} K_d	+11.2 ± 22 +13.0 ± 9	−69.3 ± 9* +325.5 ± 11*

[a]Mean ± SEM; [b]K_d and B_{max} in % of controls. Incubation (μmol . l^{-1}, 1 h): Isoprenaline (10.0), phenylephrine (1.0), trifluperazine (10.0). *Statistical significance ($P < 0.05$).

ments increased significantly (+184 ± 21%). Pretreatment of HEC with trifluperazine (Table 1) or with nonachlazine (not shown) attenuated the isoprenaline-induced increase in specific binding of (-)-S-(^{3}H)BAYK 8644, while (^{3}H)DHA binding showed a significant decrease of the B_{max} values, related to a reduced affinity of (^{3}H)DHA binding (a significant increase in the numeric values of K_d, $P < 0.05$).

Phenylephrine produced a small but significant increase in the B_{max} values of (^{3}H)DHA, and significantly reduced (-)-S-(^{3}H)BAYK 8644 binding. Exposure of HEC to isoprenaline (Table 2) induced a significant increase in the cell cAMP content. The basal adenylate cyclase activity in homogenates of HEC was 8.35 ± 0.11 pmol cAMP formed/min/mg of protein. The cell cAMP content was observed to increase significantly early after the exposure (30 s) with the cAMP levels reaching 12.2 ± 0.12 pmol. The increased cAMP content remained unaffected by pretreatment with trifluperazine or nonachlazine in other experiments. The Mg^{2+} ATP-ase activity showed increase in membrane preparations from infected as compared to control cells.

Table 2. c-AMP content of human embryonal cells exposed to isoprenaline

PROCEDURE		
BASAL (pmol c-AMP . mg^{-1} protein/min)	ISOPRENALINE (10.0 μmol . l^{-1})	
	1.0 min	60 min
CONTROL		
5.6 ± 1.2	123 ± 19*	535 ± 48*
TRIFLUPERAZINE		
3.8 ± 2.1	111 ± 23*	460 ± 68*
NONACHLAZINE		
3.9 ± 1.1	109 ± 19*	478 ± 55*

* Significant change compared to basal values ($P < 0.05$) Trifluperazine (1.0 μmol . l^{-1}), nonachlazine (20.0 μmol . l^{-1}).

Table 3. Mg^{2+} ATP-ase activity in control and infected human embryonal smooth muscle cells ($n = 6$)

BASAL (nmol Pi . min^{-1} . mg^{-1} protein)	INFECTED CELLS
6.8 ± 1.2	14.2 ± 2.9*

* Significance $P < 0.05$

Discussion

Recent evidence derived from studies on signal transduction in cardiac and smooth muscle points to the existence of direct, cAMP-dependent phosphorylation processes and also to indirect regulation of slow Ca^{2+} channels by G-protein-related events (Trautwein et al. 1990; Sperlakis and Ohya 1989). In vascular smooth muscle, cAMP is thought to mainly stimulate the Ca^{2+} uptake into the sarcoplasmic reticulum (Johns et al. 1987).

An additional evidence for the regulatory role of β-adrenergic receptor-mediated events has been obtained also in smooth muscle cell. Experiments with human vascular smooth muscle cells in culture strongly implicated the existence of a cross-regulatory communication from membrane-bound activated β-adrenergic receptors to Ca^{2+} channels (Dřímal 1989).

Clinical studies on cardiomyopathy have suggested abnormalities in the

handling of calcium by compromized muscle cells. Acute viral myocarditis has long been recognized as a precursor of the chronic dilated form of the disease. A strong support to the presence of abnormalities in the calcium handling of infected cells has also been provided by experimental studies. The chronic forms of Chaga's disease have been successfully reproduced in mice, and chronic cardiomyopathy has been demonstrated to develop after infection with Herpes simplex virus (Grodums and Zbitner 1976). Acute, as well as chronic dilated cardiomyopathy is encountered with increasing frequency in patients with the acquired immunodeficiency syndrome (Anderson et al. 1988).

It is remarkable that most humans acquire HSV by exposure at a peripheral portal, and viremia is one of the important pathogenetic modes of the virus spread (McKendall 1989). The transit of the virus from the periphery to the cortex cerebri (finding of viral RNA or DNA) with HSV is accomplished within several days (Stroop and Schaffer 1984).

Vascularized tissues, brain and heart, in addition to the cerebral and coronary vessels have been attributed to the widespread dissemination of virus. Altered contractile properties of contractile proteins or alterations in proteins regulating Ca_i^{2+} homeostasis have been proposed as the major mechanisms of functional changes of infected cells.

After development of a suitable experimental model followed by characterization of β-adrenergic and Ca^{2+} channel receptors, we therefore examined the membrane bound transduction mechanisms from β-adrenergic to Ca^{2+} channel in model of the early infection of human embryonal smooth muscle cells with a latent virus. HEC infection with the Herpes simplex virus leads to characteristic response patterns of membrane-bound β-adrenergic and Ca^{2+} channel receptors in virus-transformed cells, that is, to down-regulation of β-adrenergic receptors and to an increase in the density of "active" Ca^{2+} channels, manifested by an increased density of binding sites for the Ca^{2+} channel agonist (-)-S-(^{3}H)BAYK 8644, present on the membrane of the infected cells. Our results with (-)-S-(^{3}H)BAYK 8644, a 1.4-dihydropyridine Ca^{2+} channel activator, show clearly an increase in the numbers of "active" Ca^{2+} channels in HEC also after an exposure of the cell cultures to isoprenaline. Both experimental procedures, the virus infection and β-adrenergic receptor activation, show remarkable similarities as far as membrane receptors and enzyme activity are concerned: activation of adenylate-cyclase, down-regulation of β-adrenergic and cross-regulatory (heterologous) increase in (-)-S-(^{3}H)BAYK 8644 binding on Ca^{2+} channels.

There is considerable evidence suggesting the role of Ca^{2+}-dependent phosphorylation of smooth muscle myosin light chains as a regulatory system dominating in contraction of smooth muscle cell (Kumm and Stull 1989).

In HEC Ca^{2+} channels are probably controlled by a sophisticated loop

control mechanism. In order to substantiate the possible loop and its involvement in the generalized activation in the cells, we explored two potent phenothiazine antipsychotic agents, trifluperazine and nonachlazine, both potent inhibitors of Ca^{2+} calmodulin-sensitive systems. The results shown in Table 2 suggest that the attenuation of enhanced Ca^{2+} channel agonist binding observed after phenothiazines is probably due to inhibition of the Ca^{2+} calmodulin system in HEC. Weiss and his associates (Weiss 1980; Prozialeck et al. 1981) have reported that phenothiazines may bind irreversibly to calmodulin. Although calmodulin is primarily a cytosolic enzyme the suggestion has been made (Siekewicz et al. 1987) that changes in free Ca^{2+} concentration in the cell may control the distribution of calmodulin between membrane binding sites and the cytosol, and that Ca^{2+}-calmodulin movements as well as Ca^{2+} fluxes within the cell may be important also for coordinated regulation of cAMP-related Ca^{2+} channels.

It appears therefore that Ca^{2+}-calmodulin may be one of the indirect regulatory signals in HEC exposed to isoprenaline or infected with latent viruses.

An alternative system utilizing Ca^{2+} signal in signal transformation in smooth muscle is the membrane- bound alpha-1 adrenergic receptor (Berridge and Irvine 1984; Exton 1985). The effects of the selective alpha-1 adrenergic receptor agonist phenylephrine in our experiments with HEC showed a qualitatively different cross-regulatory response. The decreased capacity for (-)-S-(^{3}H)BAYK 8644 binding of phenylephrine pretreated HEC was result of a decreased availability of the dihydropyridine Ca^{2+} channel agonist (-)-S-(^{3}H)BAYK 8644 receptors on Ca^{2+} channels.

More recent investigations on the regulation of smooth muscle contraction have focused on the demonstration of the importance of calcium-dependent protein phosphorylation (Kumm and Stull 1989; Sellers and Adelstein 1987). Interpreted by a phosphorylation hypothesis, the phosphorylation of the membrane proteins in the light membrane fraction is associated with an increased enzyme activity in that fraction. Corresponding with these findings are also our data obtained with partially purified light membrane fraction of infected HEC showing an increased ATP-ase activity and thus an enhanced phosphorylation of membrane proteins.

In conclusion, human embryonal cells maintained in culture make a suitable cellular model for cross-regulatory studies of β-adrenergic and dihydropyridine Ca^{2+} channel receptors in smooth muscle. The β-adrenergic receptor activation with isoprenaline, as well as the early stage infection of human embryonal smooth muscle with herpes simplex virus are characterized by similarities in the membrane-bound receptor response: down-regulation of β-adrenergic receptors as result of β-adrenergic receptor activation and increase

in cAMP, and an increased capacity of dihydropyridine Ca^{2+} agonist binding on active Ca^{2+} channels. There is also an excellent correlation between the intensity of down-regulation of β-adrenergic receptors and intracellular cAMP accumulation in isoprenaline-pretreated HEC, and the observed cross-regulatory response of β-adrenergic receptors and Ca^{2+} channels after Ca^{2+}-calmodulin blockade with phenothiazines. The concept of abnormal Ca_i^{2+} and Ca^{2+} entry blockade provides an attractive rationale for the use of Ca^{2+} channel blockers in hyperdynamic contractile states associated with viremia, and possibly also in idiopathic cardiomyopathy.

References

Ahnert-Hilger G., Grabel M. (1988): Controlled manipulation of the cell interior by pore forming proteins. Trends Pharmacol. Sci. **9,** 195—198

Anderson D. M., Virami R., Reilly J. M. (1988): Prevalent myocarditist at necropsy in the acquired immunodeficiency syndrome. J. Amer. Coll. Cardiol. **11,** 792—797

Berridge M. J., Irvine R. F. (1984): Inositol-triphosphate: A novel second messenger in cellular signal transduction. Nature **312,** 315—321

Bradford M. M. (1976): A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye. Anal. Biochem. **72,** 248—254

Dřímal J. (1989): Regulation of β-adrenergic receptors and calcium channel agonist binding sites in cultured human embryonal smooth muscle cells. Gen. Physiol. Biophys. **8,** 341—350

Dřímal J., Knezl V., Magna D., Strižová K. (1987): External transport of β-adrenergic binding sites in ischemic myocardium. Gen. Physiol. Biophys. **6,** 583—591

Exton J. (1985): Role of calcium and phosphoinositides in the actions of certain hormones and neurotransmitters. J. Clin. Invest. **75,** 1753—1757

Grodums E. T., Zbitner A. (1976): Experimental herpes simplex virus carditis in mice. Infect. Immunol. **14,** 1322—1331

Hirsch M. S. (1988): Immunology and pathogenesis of persistent virus infections. In: Viral Diseases (Ed. C. Lopez), Amer. Soc. Microbiol., pp. 262—279, Washington DC

Johns A., Leiten P., Yamamoto H., Huang K., VanBreemen C. (1987): Calcium regulation in vascular smooth muscle contractility. Amer. J. Cardiol. **59,** 18A—23A

Lee T. P. (1980): Virus exposure dimishes β-adrenergic response in human Leucocytes. Res. Commun. Chem. Pathol. Pharm., **30,** 469—476

Lentz T. (1988): Binding of viral attachment protein to host-cell receptors: The Achiles heel of infectious viruses. Trends Pharmacol. Sci. **9,** 247—250

Kumm K. E., Stull J. T. (1989): Second messenger, effectors of the smooth muscle myosin phosphorylation system. Adv. Prot. Phosphatases **5,** 197—217

McKendall R. R. (1989): Herpes Simplex. In: Handbook of Clinical Neurology 12 (56) Viral Disease. (Ed. R. R. Kendall), pp. 207—227 Elsevier Sci Publ. B. V.

Oldstone M. B. (1989): Viral alteration of cell function. Sci. Amer. **261,** 42—43

Paulson J. C. (1985): Viral attachment. In: The Receptors Vol. 2. (Ed. P. M. Cortu) pp. 131—133, Academic Press. New York.

Prozialeck W. C., Cimino M., Weiss B. (1981): Photoaffinity labeling of calmodulin by phenothiazine antipsychotics. Mol. Pharmacol. **19,** 264—269

Pullmann M. E. (1967): Measurement of ^{14}C-ADP-ATP and $^{32}P_i$ATP exchange reactions. Methods in Enzymology **10,** 57—60

Sellers J. R., Adelstein R. S. (1987): The Enzymes. In: The Enzymes. (Eds. P. D. Boyer and E. G. Krebs), Vol. 18, pp. 382—418, Acad. Press, Orlando Fl., USA

Siekewicz J., Tanaka T., Saitoh M., Naka M., Inagaki M., Hidaka H. (1987): Calcium-dependent protein phospohorylation in platelet activation and the selective inhibitors. In: Platelet Activation (Ed. Yamazaki H. and Mustard J. F.), pp. 17—26, Academic Press N. Y.

Sperlakis N., Ohya Y. (1989): Regulation of Ca^{2+} channels of cardiac and vascular smooth muscle cells. Adv. Prot. Phosphatases **5,** 333—354

Stroop W. G., Schaffer B. S. (1984): Production of encephalitis related to the temporal lobes by experimental reactivation of herpes simplex virus. J. Infect. Dis. **153,** 721—731

Toews M. L. (1986): Comparison of agonist induced changes in β- and alpha-1 adrenergic receptors of DDT_1MF-2 cells. Mol. Pharmacol. **31,** 58—68

Trautwein W. Cavalie A., Allen T. J., Shuba Y. M., Pelzer S., Pelzer D. (1990): Direct and indirect regulation of cardiac L-type calcium channels by β-adrenoceptor agonists. In: Biology and Medicine of Signal Transduction, (Eds. Y. Nishizuka, M. Endo, C. Tanaka), pp. 45—50, Raven Press N. Y.

Weiss B., Prozialeck W. C., Cimino M., Barnette M. C., Wallace T. L. (1980): Acute and chronic effects of psychoactive drugs on adrenergic receptors and calmodulin. Adv. Cycl. Nucleot. Res. **12,** 213—225

Závada J., Závadová Z., Russ G., Poláková K., Rajčáni J., Štencl J., Lokša A. (1983): Human cell surface proteins selectively asembled into vesicular stomatitis virions. Virology **127,** 345—360

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