In Vivo Desensitization of Glycogenolysis to Ca^{2+}-mobilizing Hormones in Rat Liver Cells

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Abstract

Rat hepatocytes contain several types of Ca^{2+}-linked receptors, all of which stimulate glycogen breakdown by increasing cytosolic free Ca^{2+} concentration ($[Ca^{2+}]c$). In vivo desensitization of this Ca^{2+} messenger system was studied in hepatocytes isolated from either pheochromocytoma (PHEO)-harboring and chronically norepinephrine (NE)-infused rats. Homologous desensitization for $alpha_1$-adrenergic receptor-mediated phosphorylase activation developed in the early stage of PHEO rats (3–4 wk after implantation), whereas, in the later stage of tumor development or in the NE-infused rats, phosphorylase responses to all Ca^{2+}-mobilizing stimulations were subsensitive (heterologous desensitization). In the homologous desensitization, the $[Ca^{2+}]c$ response to $alpha_1$-adrenergic stimulation was selectively reduced. We found, using the phenoxybenzamine inactivation method, that there was a linear relationship between $alpha_1$ receptor density and the $[Ca^{2+}]c$ response; consequently, the blunted $[Ca^{2+}]c$ response to $alpha_1$-adrenergic stimulation could not be explained by the 34% downregulation of $alpha_1$ receptors seen in these rats. These results indicated that uncoupling at a step proximal to $alpha_1$ receptor-stimulated $[Ca^{2+}]c$ increase is also of primary importance in homologous desensitization of phosphorylase activation. On the other hand, heterologous desensitization also involved alteration(s) at steps distal to the rise in $[Ca^{2+}]c$. Our data demonstrate that prolonged exposure to catecholamines results in desensitization of the $[Ca^{2+}]c$ mobilization pathway and may involve multiple mechanisms.

Introduction

Prolonged exposure of tissues to a variety of hormones or drugs often leads to a blunted response when the tissue is subsequently exposed to the agonist. This phenomenon has been called desensitization, tolerance, or tachyphylaxis. A great deal has been learned over the past decade about the mechanism whereby receptor systems that activate adenylate cyclase may desensitize (1, 2). For example, uncoupling and downregulation of beta-adrenergic receptors frequently have been found to be associated with the desensitization of adenylate cyclase activation that occurs after prolonged exposure to catecholamines.

Much less is known about desensitization of receptors that function through another important transmembrane signaling system, namely the phosphatidylinositol (PI)[1] turnover/intracellular Ca^{2+}-mobilizing pathway. $Alpha_1$ adrenergic, vasopressin, and angiotensin II receptors are examples of receptors that function through this pathway and cause a rise of intracellular Ca^{2+} ($[Ca^{2+}]c$) in a variety of cell types (for review see references 3 and 4). The increase in the $[Ca^{2+}]c$ that occurs when these receptors are activated may explain many of the physiological effects of these receptors. Several physiological and clinical examples exist that suggest that this signal transduction system also undergoes adaptive changes in the face of continued stimulation (5–9).

The liver is a major site of glycogen deposition and breakdown in the regulation of mammalian glucose homeostasis, and the balance between these processes is regulated by a variety of stimulators, including Ca^{2+}-mobilizing hormones. In particular, the regulation of glycogenolysis by Ca^{2+}-linked hormones has been extensively studied in adult male rat liver, in which glycogen phosphorylase, the key enzyme for this process, is mainly regulated by Ca^{2+}-mobilizing hormones. Its biochemical framework is also well established (for review see references 3, 4, 10, and 11); thus, the available experimental evidence indicates that an elevation of $[Ca^{2+}]c$ after the occupation of specific receptors by these Ca^{2+}-mobilizing hormones allosterically activates phosphorylase b kinase (12) and results in an increased rate of glycogen breakdown. Several in vitro studies involving an examination of desensitization of glycogen phosphorylase activation by Ca^{2+}-mobilizing hormones (13, 14) have used this model system of isolated rat hepatocytes. However, very little information is available on in vivo desensitization of responses mediated by Ca^{2+}-mobilizing hormones.

In these studies, we have investigated the consequences of prolonged exposure to catecholamines on the activation of hepatic glycogenolysis to Ca^{2+}-mobilizing hormones. High circulating levels of catecholamines were maintained in rats either by transplantable catecholamine-producing tumor pheochromocytoma (PHEO) or by subcutaneously implanted osmotic minipumps. We found that desensitization of hepatic glycogenolysis to Ca^{2+}-mobilizing hormones can occur in in vivo settings. Experiments in hepatocytes isolated from rats chronically exposed to catecholamines revealed multiple mechanisms in the process of desensitization.

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1. *Abbreviations used in this paper:* Bt_2cAMP, dibutyryl cAMP: $[Ca^{2+}]c$, cytosolic free calcium concentration; NE, norepinephrine; NEDH, New England Deaconess Hospital; PHEO, pheochromocytoma; PI, phosphatidylinositol; quin2/AM, quin2-tetraacetyl oxymethyl ester.

Methods

Materials

Chemicals were obtained from the following sources: Quin2-tetraacetoxymethyl ester (quin2/AM), Dojindo Laboratories, Kumamoto, Japan; type II collagenase, Worthington Biochemicals, Freehold, NJ; (−) phenylephrine HCl, angiotensin II, (±) propranolol HCl, Arg-vasopressin, glucagon, dibutyryl cAMP (Bt_2cAMP), and Hepes, Sigma Chemical Co., St. Louis, MO; forskolin and A23187, Calbiochem-Behring Corp., La Jolla, CA. [^{3}H]Prazosin (specific activity, 19.8 Ci/mmol) and alpha-D-[*U*-^{14}C]glucose 1-phosphate were from New England Nuclear (Boston, MA). Aequorin was purchased from Dr. J. R. Blinks of the Mayo Foundation (Rochester, MN) as 1 mg lyophilized protein in KCl and 1,4-piperazine-bis(ethanesulfonic acid) buffer. Phenoxybenzamine HCl was generously supplied by SmithKline & French Laboratories (Philadelphia, PA). All other chemicals and reagents used were from standard commercial sources.

Animal model of PHEO

8–9-wk-old male New England Deaconess Hospital strain (NEDH) rats were fed standard laboratory chow ad lib. and maintained on a 12-h light/dark (0600/1800 h) cycle. Rats were lightly anesthetized with ether and implanted subcutaneously at the base of the neck with several pieces (≅1 × 1 × 3 mm) of rat PHEO taken from another NEDH rat harboring the tumor. Several tumor-bearing rats (line P-259 established by Warren and Chute, reference 15) were generously provided by Dr. William M. Manger, New York University Medical Center, New York, NY. Age-matched male NEDH controls were also lightly anesthetized with ether and received only an intrascapular skin incision. After the surgery, rats were housed in individual cages with free access to food and water. A palpable tumor mass was generally evident 3–4 wk after tumor implantation; the tumor was ~ 0.5–0.8 cm in diameter at this time. The animals' body weight also served as an indication of the progression of the tumor (15–17). Tumor-bearing animals gained weight at a rate similar to unimplanted age-matched controls for several weeks after tumor implantation. By the time the tumor was palpable, the body weight generally plateaued for 4–7 d and then rapidly decreased. The tumor-bearing animals used in this study exhibited body weight plateaus at 21–28 d after tumor implantation. Animals were studied either when their body weight reached a plateau (early stage; 3–4 wk after tumor implantation) or when a rapid weight loss was apparent (late stage; 6–7 wk after tumor implantation); age-matched unimplanted controls were studied at the same time (17).

Infusion of norepinephrine (NE) in vivo

To examine whether the altered responsiveness observed in rats with PHEO was attributable solely to elevation of plasma NE or might be related to the presence of the tumor and associated changes in tumor-bearing animals, we compared changes using subcutaneously implanted osmotic minipumps to infuse NE. To raise circulating levels of NE in vivo, we implanted osmotic minipumps (Alzet; Alza Corp., Palo Alto, CA) subcutaneously on the backs of male NEDH rats. All rats used were 11–12 wk old and weighed ~ 280–300 g at the time of minipump implantation. The pumps, which are designed to infuse the drug continuously for up to 14 d at a constant rate of 500 μg/kg per h, were filled with NE in 0.001 N HCl; control rats were implanted with minipumps containing the vehicle (0.001 N HCl) alone. The rats were examined 24 h, 2 d, 4 d, or 7 d after implantation of the minipumps.

Analysis of plasma catecholamine concentrations

To analyze plasma catecholamine concentrations, blood (1 ml) from the inferior vena cava below the level of the renal veins was rapidly collected through an abdominal incision just before starting the liver perfusion. The blood was placed in ice-cold tubes containing 0.1% sodium EDTA as an anticoagulant. After centrifugation at 1,500 *g* for 10 min, the plasma samples were stored at −80°C until analyzed. Plasma samples were extracted as previously described (18). Catecholamine determinations were made by reverse-phase HPLC with electrochemical detection as described previously (19) using the 88:12 (vol/vol) mixture of 0.1 M potassium phosphate buffer (pH 3.2) and methanol with 2.5 mM octane sulfonate sodium and 10 μM sodium EDTA as the mobile phase.

Preparation of hepatocytes

Isolation of hepatocytes was routinely initiated 3–4 h into the light period. Isolated hepatocytes were prepared by a modification (20) of the collagenase perfusion method of Berry and Friend (21) as described previously (22). Isolated hepatocytes from an individual rat were then divided for the measurement of phosphorylase a and [Ca^{2+}]c, and for radioligand binding studies. The yield of cells from one liver averaged 1.5×10^8, with 90–95% viability as estimated by trypan blue exclusion.

Fractional inactivation of alpha$_1$ receptors by phenoxybenzamine

In some experiments to delineate the linkage between alpha$_1$-adrenergic receptor occupation and [Ca^{2+}]c response, we used the phenoxybenzamine inactivation method (7, 23, 24). In these experiments, to irreversibly inactivate alpha$_1$-adrenergic receptors, phenoxybenzamine was added to the hepatocytes suspension in final concentrations ranging from 10^{-10}–10^{-5} M and was incubated for 20 min at 37°C. Hepatocytes were then washed three times with the buffer used for the radioligand binding assay and for [Ca^{2+}]c measurements. Data are expressed as percent of control; thus, for receptor occupation, B/B_{max}, where B is the amount of [^{3}H]prazosin specifically bound under the experimental conditions and B_{max} is the amount of specific [^{3}H]-prazosin binding in the absence of any phenoxybenzamine, and for [Ca^{2+}]c measurements, as percent of the amplitude of aequorin bioluminescence response or that of peak [Ca^{2+}]c measured by quin2 seen with 100 μM phenylephrine alone.

Analytical procedure

Glycogen phosphorylase a activity. Glycogen phosphorylase a activity was assayed in liver-cell homogenates prepared as described previously (22). Unless otherwise stated, cell samples were taken for determination of phosphorylase a activity 2 min after hormone additions. Glycogen phosphorylase a activity was measured using a filter disc assay similar to that of Gilboe et al. (25) as modified (26). Units of phosphorylase activity are micromoles of [^{14}C]glucose from alpha-D-[*U*-^{14}C]glucose 1-phosphate incorporated into total assay glycogen per min per 100 mg of protein.

Measurement of [Ca^{2+}]c in rat hepatocytes. Measurement of [Ca^{2+}]c in isolated hepatocytes was achieved by using either the photoprotein aequorin and the fluorphore quin2.

[Ca^{2+}]c measurement (aequorin). Incorporation of aequorin into isolated hepatocytes was performed by the gravity loading method described by Borle et al. (27) with modifications. The aequorin-loaded cells were resuspended and incubated at 37°C in modified Hanks' buffer containing (in millimolar) NaCl, 137; KCl, 3.5; KH_2PO_4, 0.44; $NaHCO_3$, 4.2; Na_2HPO_4, 0.33; $CaCl_2$, 1.0; $MgCl_2$, 1.0; glucose, 15; and 0.5% (wt/vol) dialyzed BSA. Bioluminescence was measured by a modified photomultiplier tubeaggregometer (lumiaggregometer; Chrono-Log Corp., Havertown, PA) under constant stirring at 37°C. Resting [Ca^{2+}]c was calculated from a calibration curve as previously published by Borle and Snowdowne (28, 29), assuming the even distribution of calcium in the cell. [Ca^{2+}]c in stimulated cells was not calibrated, because no information was available as to the spatial distribution of calcium in the cell. The magnitude of aequorin luminescence is expressed as electric current. None of the drugs used in this experiments quenched luminescence of aequorin added to a cell-free suspension. Our preliminary experiments confirmed that there was no difference between aequorin-loaded and -unloaded hepatocytes in the glycogen phosphorylase responses induced by 100 μM phenylephrine, 10 μM epinephrine, 100 mIU/ml Arg-vasopressin, and 100 nM angiotensin II.

In some experiments, to determine the efficiency of aequorin incorporation, aequorin content was determined in an aequorin assay photometer by measuring the light signal emitted from lysed cells in the presence of a saturating concentration of $CaCl_2$ (28, 29). The resultant flash of light was recorded as current from the photomultiplier tube and normalized by the cell protein concentration.

$[Ca^{2+}]c$ measurement (quin2). Quin2 loading was achieved essentially as reported by Thomas et al. (30). The cells were suspended in Hepes buffer containing (in millimolars) NaCl, 120; Hepes, 20; KCl, 5.4; $NaHCO_3$, 4.2; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 1.0; glucose, 15; and 2.0% (wt/vol) dialyzed BSA at pH 7.4 and maintained under an atmosphere of O_2/CO_2 (95:5%). After 20 min incubation with 100 μM quin2/AM, when maximal accumulation of quin2 had occurred, the cells were washed twice and resuspended, at ~ 3 mg of dry weight/ml in the Hepes buffer described above with the albumin concentration reduced to 0.2%. Quin2 fluorescence measurements were performed in a fluorescence spectrophotometer (model F-3000; Hitachi Ltd., Tokyo, Japan) equipped with a thermostat-controlled cuvette holder and magnetic stirring. Excitation and emission wavelengths were 340 and 490 nm, respectively. Calibration and determination of $[Ca^{2+}]c$ were done as described by Tsien et al. (31).

$[^3H]$Prazosin binding. [^{3}H]Prazosin binding experiments were performed on a crude cellular homogenate as previously described (32). Saturation binding experiments with [^{3}H]prazosin were carried out at a range of 0.5–5 nM.

Protein concentration was determined by the method of Lowry et al. (33) using BSA as standard.

Data analysis. All data for each drug were meaned and the resulting concentration-response curves from each group were analyzed simultaneously using the four-parameter logistic equation (34). The resulting ED_{50} and maximal responses (E_{max}) were analyzed for significant differences using the ALLFIT program. The ALLFIT program is a modification of the DeLean et al. program by Martin H. Teicher, and was obtained from the Biomedical Computing Technology Information Center, Nashville, TN. Data derived from radioligand studies were analyzed by LIGAND, a nonlinear computer-assisted iterative weighed least square curve-fitting procedure (35).

The experimental data given in the text and figures are the means ±SEM of n experiments as indicated. Differences between means within each experiment were evaluated by analysis of variance. If analysis of variance demonstrated a significant difference among means, the t test for unpaired observations was then used to determine which pairs of means were significantly different. The criterion for statistical significance was a P value of < 0.05. In several figures in which no significant difference was obtained either between the younger and older age-matched control NEDH rats or between early and late stage of PHEO rats, these two groups of data were pooled; however, the statistical comparison of responses obtained from either early or late stage of PHEO rats was always made with the corresponding age-matched controls.

Results

Effect of alpha$_1$-adrenergic stimulation on phosphorylase activity and $[Ca^{2+}]c$ response. Fig. 1 shows the time course for phosphorylase activation and $[Ca^{2+}]c$ signals indicated either by quin2 or aequorin after addition of the alpha$_1$-adrenergic agonist phenylephrine (100 μM) to isolated hepatocytes. Significant phosphorylase activity was observed 5 s after phenylephrine addition, and this approached a maximum value after 15–20 s, which is kept by 3 min (Fig. 1 *A*). The time course of $[Ca^{2+}]c$ signals seen in response to phenylephrine differed in aequorin- and quin2-loaded hepatocytes (Fig. 1, *B* and *C*). The aequorin signals displayed a more rapid rise in luminescence to a peak followed by a fall to baseline that may represent

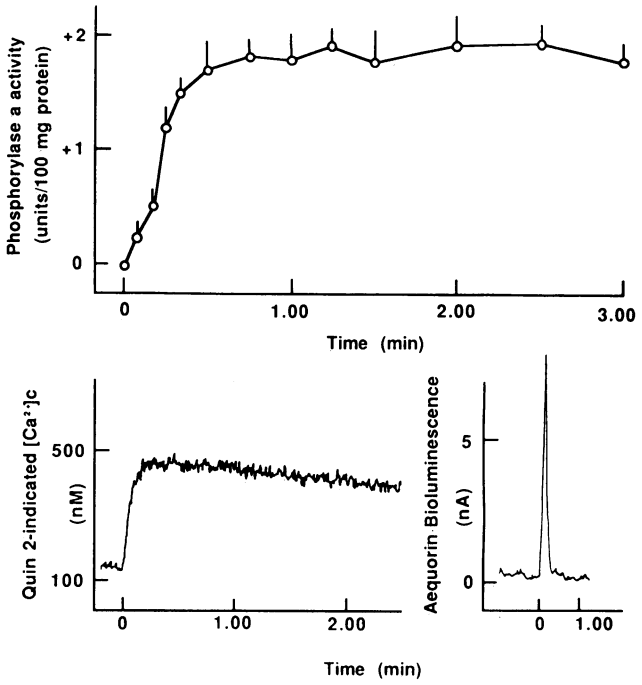

Figure 1. Time courses of the changes in phosphorylase activation (*top*), and cytosolic free Ca^{2+} concentration indicated by aequorin (*lower right*) and by quin2 (*lower left*) in response to 100 μM phenylephrine in isolated hepatocytes. Phosphorylase activation: 5 ml hepatocytes were incubated with phenylephrine, and samples were removed for determination of phosphorylase a at the time shown. Values shown are means±SEM of triplicate incubations from a representative experiment of three. $[Ca^{2+}]c$ increase: aequorin- and quin2-loaded hepatocytes were prepared as described in Methods. Aequorin bioluminescence and quin2 fluorescence measurements were collected sequentially and every 0.5 s, respectively. The results presented are representative experiments of at least five experiments.

either decline in $[Ca^{2+}]c$ or aequorin consumption. However, the transient nature of the aequorin signal is not due to consumption of aequorin, since subsequent addition of a different agonist resulted in a prompt increase in the light; also, intracellular aequorin content measured by lysing the cells using Triton X-100 was not decreased significantly after a single addition of maximal dose of any of the agonists (data not shown). Quin2, on the other hand, demonstrated a similarly brisk upstroke to a peak, but followed by a plateau and a very slow decline in the signal, whose time course apparently corresponds well to that of phosphorylase activation.

In aequorin-loaded cells, resting $[Ca^{2+}]c$ of the rat hepatocytes was 148±16 nM (n = 8), assuming intracellular magnesium concentration of 1 mM and even distribution of calcium in the cell. In quin2-loaded cells, 100 μM phenylephrine caused an increase in $[Ca^{2+}]c$ from a basal level of 175±14 nM (n = 12) to a peak of 495±34 nM (n = 8). In a series of preliminary experiments we observed an appropriate concentration-$[Ca^{2+}]c$ response relationship for several Ca^{2+}-mobilizing hormones (phenylephrine, epinephrine, arg-vasopressin, and angiotensin II) in both aequorin luminescence and quin2 fluorescence. Also, using selective antagonists we observed that both aequorin- and quin2-indicated $[Ca^{2+}]c$ responses to catecholamines occurred via alpha$_1$ receptors (data not shown).

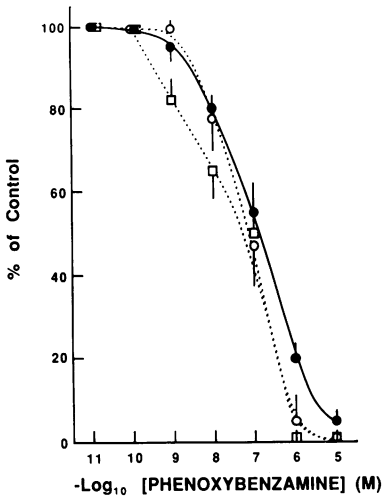

Figure 2. Effect of a 20-min incubation of isolated rat hepatocytes with several concentrations of phenoxybenzamine on maximal [^{3}H]-prazosin binding capacity (●) and 100 μM phenylephrine-induced changes in cytosolic free Ca^{2+} concentration indicated either by aequorin (○) or by quin2 (□). Data are means±SEM of five [^{3}H]prazosin binding or at least four aequorin bioluminescence or quin2 fluorescence experiments.

Effects of receptor inactivation with phenoxybenzamine on [^{3}H]prazosin binding and phenylephrine-stimulated [Ca^{2+}]c response. After a 20-min incubation of freshly isolated hepatocytes with various concentrations of phenoxybenzamine, freshly isolated hepatocytes were extensively washed, and the extent of receptor inactivation was determined by [^{3}H]-prazosin binding to the remaining receptor sites. Maximum [^{3}H]prazosin binding capacity was progressively decreased by exposure to increasing concentrations of phenoxybenzamine, reaching a maximal reduction of 94±3% at a phenoxybenzamine concentration of 10 μM (n = 5), and the phenoxybenzamine concentration necessary to block half of the [^{3}H]prazosin binding sites in 20 min is 119±46 nM (n = 5) (Fig. 2). Also, the effect of partial $alpha_1$ receptor inactivation on 100 μM phenylephrine-stimulated [Ca^{2+}]c response as assessed by quin2 and aequorin is shown in Fig. 2. The half-maximal inhibitory concentrations (IC_{50}) for 100 μM phenylephrine-stimulated [Ca^{2+}]c response are 69±18 (n = 5) and 78±58 nM (n = 4) phenoxybenzamine for aequorin and quin2 signals, respectively. Correlation of fractional inhibition of phenylephrine-induced [Ca^{2+}]c responses detected by aequorin and quin2 to fractional inhibition of [^{3}H]prazosin binding approximated a unitary relationship in each case. The correlation of inhibition of phenylephrine-induced [Ca^{2+}]c response detected by aequorin and [^{3}H]prazosin binding has a slope of 1.13 and a correlation coefficient of 0.98. The slope of the line formed by a corresponding analysis of inhibition of phenylephrine-induced [Ca^{2+}]c response detected by quin2 is 1.07, and the correlation coefficient is 0.97. Thus, a close correlation between $alpha_1$-adrenergic receptor occupancy and the functional response of [Ca^{2+}]c can be demonstrated in isolated rat hepatocytes.

Studies in rats harboring PHEO. Table 1 summarizes the body weight and the concentration of plasma catecholamines in either the PHEO-bearing (both early and late stages) rats compared with age-matched, unimplanted NEDH controls. As we found previously (16), the body weights of tumor harboring (PHEO) and control rats were similar at ~ 4 wk after tumor implantation (early stage), whereas a marked loss of body weight was noted in PHEO rats at 7–8 wk after tumor transplant (late stage). There was a marked increase in the plasma levels of dopamine and NE in rats with the tumor irrespective of their stages compared with controls, whereas plasma epinephrine levels were not significantly altered (Table I). The high concentrations of plasma catecholamines were not correlated with the disease stage or tumor size. However, the tumors weighed significantly more in the late stage compared with the early stage. The tumors were usually solitary, highly vascular, and well encapsulated. In larger tumors (weighing > 2 g), areas of necrosis and fibrosis were generally observed. No metastases were visually evident.

Glycogen phosphorylase activity was examined in hepatocytes isolated from both stages of PHEO rats. The basal levels of the glycogen phosphorylase activity in cells isolated from PHEO rats were similar to those of age-matched controls. In the presence of 15 mM glucose-1-phosphate, the activities were 0.96±0.21 U/100 mg protein (n = 11) vs. 1.12±0.18 U/100 mg protein (n = 9) for the early stage of PHEO rats and age-matched controls; 1.21±0.22 U/100 mg protein (n = 10) vs. 1.08±0.24 U/100 mg protein (n = 9) for the late stage of PHEO rats and age-matched controls, respectively. The abilities of alpha-adrenergic agonists, phenylephrine and epinephrine, to activate glycogen phosphorylase in control and both stages of PHEO rats are presented in Fig. 3 *A*. In both early and late stages of PHEO rats, the phosphorylase activation was markedly decreased at all concentrations examined compared with that of age-matched controls.

Further experiments were performed to determine if the desensitization in responsiveness to alpha-adrenergic agonists was homologous or heterologous. In this case, homologous desensitization would involve loss of responsiveness only to catecholamines, whereas responsiveness to drugs or hormones causing their action through other receptors would not be impaired (36). Conversely, heterologous desensitization involves

Table I. Comparison of Control and PHEO-implanted Rats

	Control NEDH rats (11–12-wk old)	PHEO-bearing rats (Early stage)	Control NEDH rats (14–15-wk old)	PHEO-bearing rats (late stage)
Body weight (*g*)	288±4 (n = 16)	278±6 (n = 11)	348±3 (n = 14)	224±3* (n = 12)
Tumor weight (*g*)	—	1.1±0.2 (n = 9)	—	5.3±0.4‡ (n = 8)
Plasma dopamine (*pg/ml*)	168±42 (n = 8)	33,300±9,200* (n = 7)	188±32 (n = 8)	41,600±12,700* (n = 8)
Plasma NE (*pg/ml*)	198±30 (n = 8)	28,400±5,440* (n = 7)	201±58 (n = 8)	22,300±4,720* (n = 8)
Plasma epinephrine (*pg/ml*)	128±22 (n = 8)	156±46 (n = 7)	108±29 (n = 8)	185±59 (n = 8)

* $P < 0.01$ vs. age-matched control NEDH rats; ‡$P < 0.01$ vs. early-stage PHEO-bearing rats; no symbol indicates no significant difference ($P > 0.05$). Plasma catecholamine measurements were done as described in Methods. Values are mean±SEM; n refers to the number of rats.

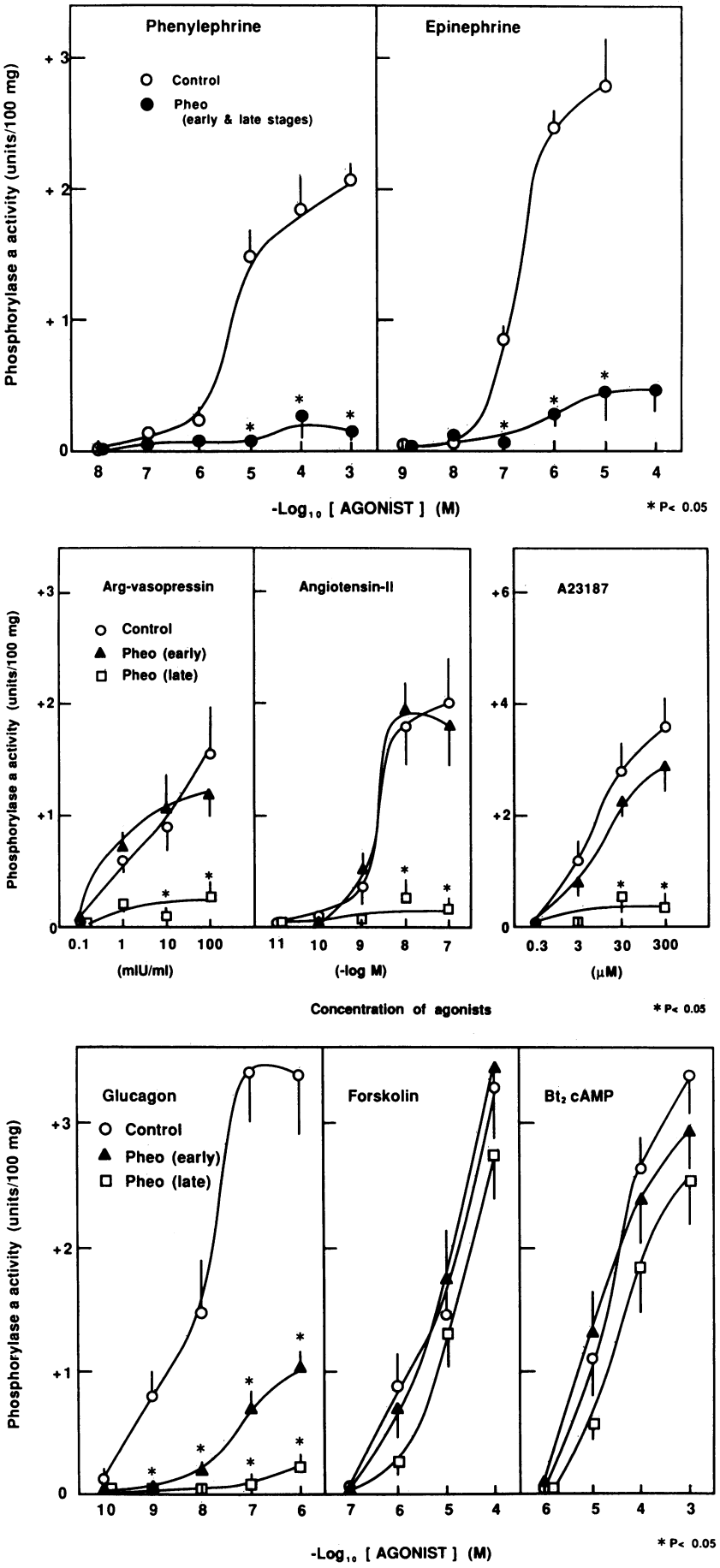

Figure 3. (*A*) Activation of glycogen phosphorylase to phenylephrine (*left*) and epinephrine (*right*) in isolated rat hepatocytes. Three groups of animals were studied: age-matched controls, early stage, or late stage of PHEO-bearing rats. As no significant difference was obtained either between the younger and older age-matched controls or between the early and late stages of PHEO rats, these two groups of data were pooled. Each point with a vertical bar represents the mean±SEM of 12–15 experiments. (*B*) Activation of glycogen phosphorylase to Arg-vasopressin, angiotensin-II, and the Ca^{2+} ionophore A23187 in isolated rat hepatocytes. Three groups of animals were studied: age-matched control (○), early stage (▲), and late stage (□) of PHEO-bearing rats. Each point with a vertical bar represents the mean±SEM of five to seven experiments. (*C*) Activation of glycogen phosphorylase to glucagon, forskolin, and Bt_2cAMP in isolated rat hepatocytes. Three groups of animals were studied: age-matched control (○), early-stage (▲), and late-stage (□) of PHEO-bearing rats. Each point with a vertical bar represents the mean±SEM of at least four experiments. *$P < 0.05$ difference from controls.

loss in responsiveness to a multiplicity of agonists acting on different receptors (36). To address this issue, we have examined the phosphorylase activation to the two nonadrenergic hormones, arg-vasopressin and angiotensin II, and the Ca^{2+} ionophore A23187. As shown in Fig. 3 *B*, in the early-stage PHEO rats, glycogen phosphorylase activation of hepatocytes by these Ca^{2+}-mobilizing stimuli was similar to controls; whereas, in the late-stage PHEO rats, at all concentrations examined the phosphorylase responses were significantly ($P < 0.05$) smaller compared with those of controls. Phosphorylase activation by stimulations that cause an increase in cAMP was also shown in Fig. 3 *C*. In both early and late stages of PHEO rats, phosphorylase activation by Bt_2cAMP and forskolin in these rats were not significantly different from those of controls, whereas glucagon-stimulated phosphorylase activation was found to be significantly lower in both stages of PHEO rats than in age-matched control rats.

In an effort to determine the mechanism for the desensitization of hepatic phosphorylase activation to Ca^{2+}-mobilizing hormones in rats harboring PHEO, $[Ca^{2+}]c$ responses in hepatocytes to 100 μM phenylephrine, 10 mIU/ml arg-vasopressin and 10 nM angiotensin II were measured in both early and late stages of PHEO rats. Estimated resting $[Ca^{2+}]c$ values in the presence of 1 mM extracellular Ca^{2+} for hepatocytes of PHEO rats (aequorin-indicated $[Ca^{2+}]c$ were 181±26 nM, $n = 8$ and 197±31 nM, $n = 7$; quin2-indicated $[Ca^{2+}]c$ were 154±22 nM, $n = 9$ and 186±28 nM, $n = 7$ for the early and late stage of PHEO rats, respectively) were similar to those of age-matched controls (aequorin-indicated $[Ca^{2+}]c$ were 162±26 nM, $n = 6$ and 171±33 nM, $n = 6$; quin2-indicated $[Ca^{2+}]c$ were 197±34 nM, $n = 5$ and 178±30 nM, $n = 5$ for the early and late stage of controls, respectively). As shown in Fig. 4, both aequorin- and quin2-indicated $[Ca^{2+}]c$ signals induced by 100 μM phenylephrine were greatly decreased in the early stage of PHEO rats, and were abolished in the late stage of PHEO rats. However, the $[Ca^{2+}]c$ responses to arg-vasopressin and angiotensin II detected by both aequorin and quin2 methods were not significantly different from controls in the early stage of PHEO. With the $[Ca^{2+}]c$ responses to arg-vasopressin and angiotensin II in the late stage of PHEO rats, the results obtained by aequorin and quin2 were found to be different; aequorin-indicated $[Ca^{2+}]c$ signals were not significantly different from those seen in controls, whereas quin2-indicated ones were markedly decreased.

The results of $[Ca^{2+}]c$ responses suggested that homologous desensitization of hepatic glycogen phosphorylase activation to alpha adrenergic stimulation seen in the early stage of PHEO rats may be closely related to the change proximal to $[Ca^{2+}]c$ changes. Thus, we next examined the possibility that an alteration in $alpha_1$ receptor number or affinity might be associated with the homologous desensitization. Saturation binding experiments performed with hepatocytes membrane preparations from rats with PHEO rats (early stage) showed 34% decrease in total [^{3}H]prazosin binding (100.4±10.8 fmol/mg protein, $n = 5$ in control rats vs. 66.2±8.5 fmol/mg protein, $n = 5$ in PHEO rats, $P < 0.05$; Fig. 5, *left*) without any significant change in the affinity (K_d = 0.14±0.04 nM, $n = 5$ in control rats vs. K_d = 0.08±0.02 nM, $n = 5$ in PHEO rats). In addition, the affinity of the $alpha_1$-adrenergic receptors for (−)epinephrine was not significantly different in hepatocyte membrane preparations from PHEO rats when the affinity was determined in competition experiments with [^{3}H]prazosin (Fig. 5, *right*). The half-maximal effective concentration (EC_{50}) in controls was 38±9 μM, $n = 5$ vs. 44±12 μM, $n = 5$ in PHEO rats. The lack of change in $alpha_1$ receptor affinity for the radioligand indicates that circulating catecholamines were not retained on receptors from animals with PHEO; retention of the agonist would be expected to decrease the affinity of the receptor for [^{3}H]prazosin.

Studies in rats infused with NE. Table II summarizes the body weight change and the concentration of plasma catecholamines in rats infused with NE by subcutaneously implanted osmotic minipumps. Infusion of 500 μg/ml per h NE increased plasma NE 30–50-fold with no change in the level of dopamine or epinephrine. The elevated NE concentration, however, was ~ two to five-fold smaller compared with those of

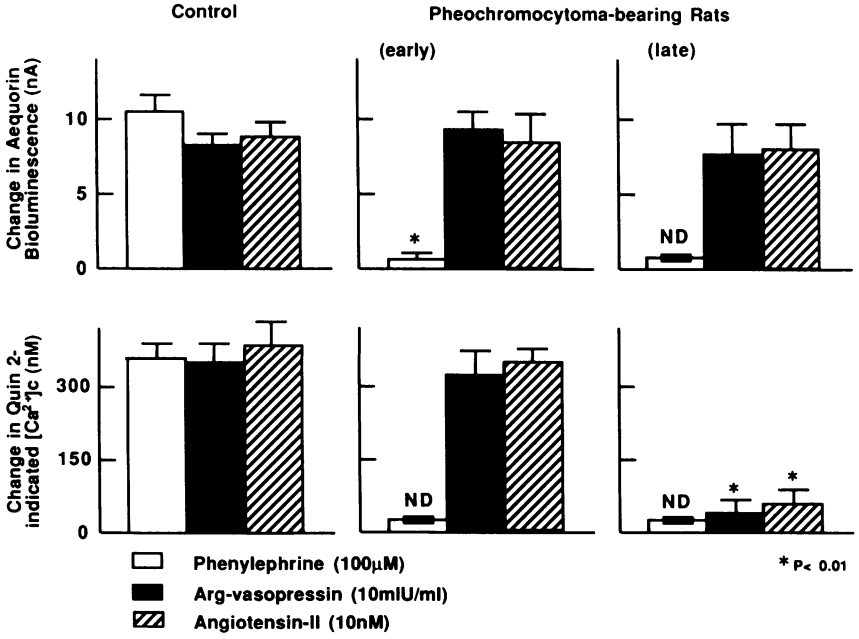

Figure 4. Effects of PHEO on phenylephrine-, Arg-vasopressin-, and angiotensin II-induced changes in cytosolic free Ca^{2+} concentration in isolated rat hepatocytes. Three groups of animals were studied: age-matched controls (*left*), or early-stage (*middle*), late-stage (*right*); of PHEO rats in medium containing 1.0 mM extracellular calcium. Values shown are the mean±SEM (*bars*) of five to six experiments. *$P < 0.01$ difference from controls.

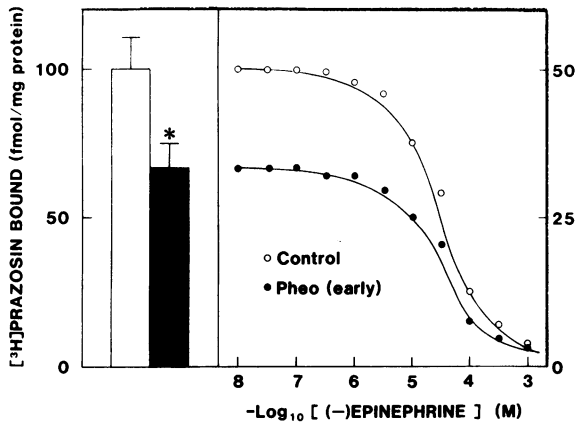

Figure 5. (*Left*) Changes in alpha$_1$-adrenergic receptors in hepatocyte membranes from the age-matched control rats (*open bar*) and the early stage of pheochromocytoma-bearing rats (*closed bar*). Values shown are maximal number of receptor sites (mean±SEM). The affinities of alpha$_1$-receptors for [^{3}H]prazosin were not different in the two groups. *$P < 0.05$ difference from controls. (*Right*) Concentration-effect curves for inhibition of [^{3}H]prazosin binding by (−) epinephrine in hepatocyte membranes from control (○) and the early stage of PHEO-bearing rats (●). The curves are the mean of five experiments performed in duplicate.

PHEO rats. This extent of increase was detectable by 14 h (the earliest time point tested) and remained at this level for the duration of the infusion (data not shown). Corresponding to the increase of NE, the body weight progressively decreased over the time of the infusion.

Hepatic phosphorylase was examined on 1–2, and 4–7 d of NE infusion. The basal level of the glycogen phosphorylase activity was slightly decreased in hepatocytes isolated from NE-infused rats. The activities were 0.97±0.20 U/100 mg protein (n = 11) for control, 0.68±0.18 (n = 8) and 0.78±0.21 (n = 11) U/100 mg protein for 1–2 d and 4–7 d of NE-infused rats, respectively. The abilities of Ca^{2+}-mobilizing hormones and cAMP-elevating agents to activate glycogen phosphorylase in control and NE-infused rats are presented in Fig. 6. In both groups of NE-infused rats, the phosphorylase activation to all Ca^{2+}-linked stimulations was markedly decreased at all concentrations examined compared with the controls (Fig. 6, *A* and *B*); this attenuation was observed by 24 h of NE infusion, suggesting that the heterologous desensitization develops very rapidly by infusion of NE, in contrast to the situation in PHEO rats. In these rats, phosphorylase activations by Bt_2cAMP and forskolin were not significantly different from those of controls; however, phosphorylase activation by glucagon was significantly ($P < 0.05$) lower than in control rats (Fig. 6 *C*).

Estimated resting [Ca^{2+}]c values in the presence of 1 mM extracellular Ca^{2+} for hepatocytes of NE-treated rats (aequorin-indicated [Ca^{2+}]c were 202±21 nM, n = 5 and 218±31 nM, n = 8; quin2-indicated [Ca^{2+}]c were 184±29 nM, n = 5 and 197±47 nM, n = 5 for 1–2 days and 4–7 d infusion of NE, respectively) were not significantly different from those values of controls (aequorin-indicated [Ca^{2+}]c was 165±27 nM, n = 7; quin2-indicated [Ca^{2+}]c was 170±21 nM, n = 12). As shown in Fig. 7, 100 μM phenylephrine-induced changes in both aequorin- and quin2-indicated [Ca^{2+}]c are significantly ($P < 0.05$) reduced after 1–2 d of NE infusion and became nondetectable after 4–7 d of infusion. Similar to findings in the late stage of PHEO rats, rises in [Ca^{2+}]c to arg-vasopressin and angiotensin II were found to be different between aequorin- and quin2-indicated signals. In hepatocytes treated by NE infusion for 1–2 d, aequorin-indicated [Ca^{2+}]c signals were not significantly different from those in controls, whereas quin2-indicated ones were significantly decreased. Longer infusion of NE (4–7 d) was found to result in a more general reduction in both aequorin- and quin2-indicated [Ca^{2+}]c signals; thus, quin2-indicated rises in [Ca^{2+}]c were not detectable in these cells.

We further examined the role of the alpha$_1$ receptors in the rapid development of the reduction in [Ca^{2+}]c response to alpha$_1$-adrenergic stimulation observed in hepatocytes from NE-infused rats for 1–2 d. Saturation binding experiments performed with hepatocytes membrane preparations from rats with NE infusion (1–2 d) showed that the number of hepatic alpha$_1$-adrenergic receptors was slightly decreased, but the observed decrease from control was not significant at the 5% level (98.3±10.6 fmol/mg protein, n = 5 in control rats vs. 79.5±9.6 fmol/mg protein, n = 5 in NE-infused rats), nor were the affinities of the alpha$_1$-adrenergic receptors for [^{3}H]prazosin in hepatic membranes different between control rats and rats with NE infusion (K_d = 0.17±0.05 nM, n = 5 in control rats vs. K_d = 0.13±0.02 nM, n = 5 in NE-treated rats).

Discussion

Using the glycogenolysis in the rat liver as a model system, we have examined in vivo desensitization of the Ca^{2+} messenger system. In both PHEO-harboring and chronically NE-infused rats, we found two different patterns of desensitization in the ability of Ca^{2+}-mobilizing hormones to activate hepatic phosphorylase. In homologous desensitization for alpha$_1$-adrener-

Table II. Comparison of Control and NE-infused Rats

	Control NEDH rats (11–12-wk old)	NE-infused rats	
		1–2 d	4–7 d
Body weight (*g*)	295±4 (n = 10)	269±6* (n = 9)	226±5*‡ (n = 9)
Plasma dopamine (*pg/ml*)	162±36 (n = 8)	133±32 (n = 7)	101±28 (n = 8)
Plasma NE (*pg/ml*)	172±34 (n = 8)	5,530±2,010* (n = 7)	9,200±3,400* (n = 8)
Plasma epinephrine (*pg/ml*)	119±30 (n = 8)	168±46 (n = 7)	128±65 (n = 8)

* $P < 0.01$ vs. age-matched control NEDH rats; ‡$P < 0.05$ vs. NE-infused (1–2 d) rats; no symbol indicates no significant difference ($P > 0.05$). Plasma catecholamine measurements were done as described in Methods. Values are mean±SEM; n refers to the number of rats.

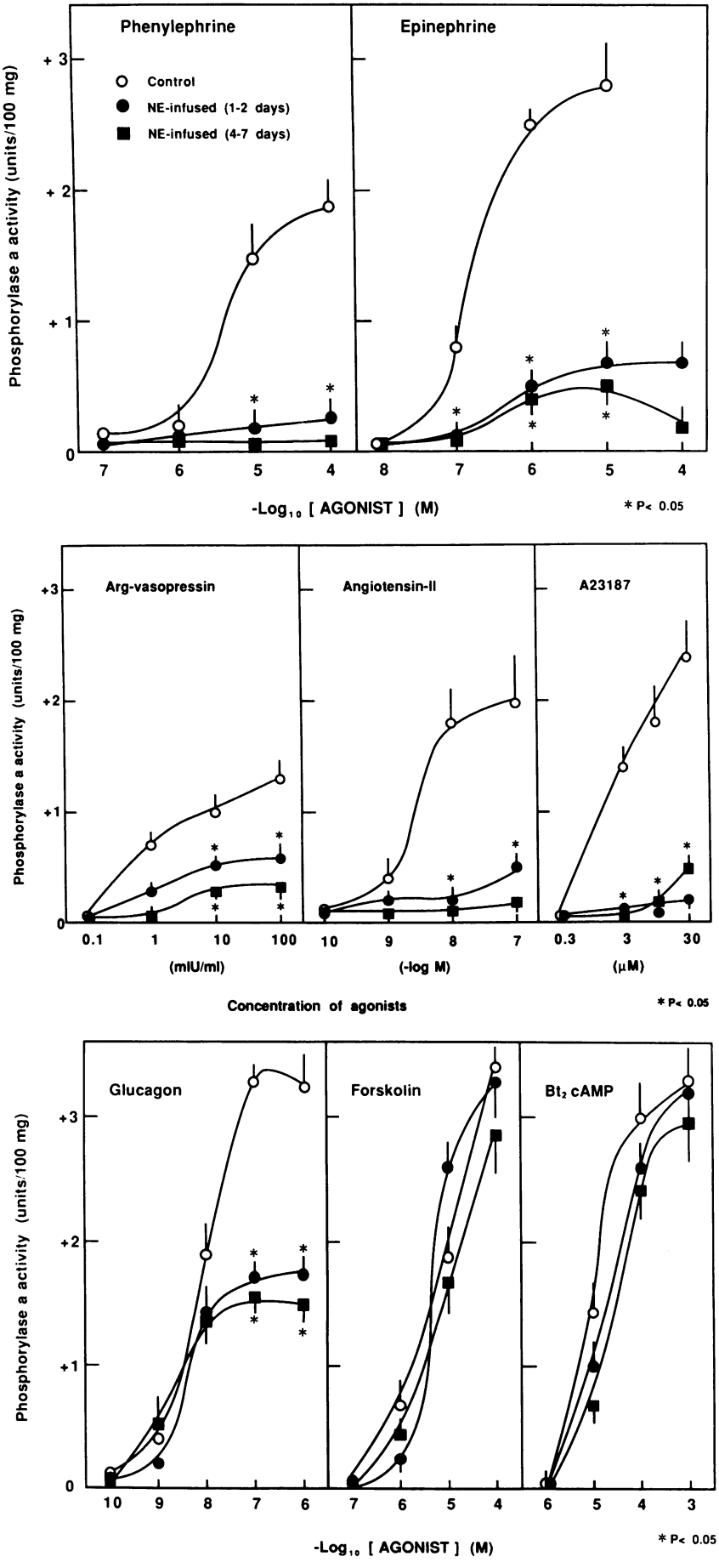

Figure 6. (*A*) Activation of glycogen phosphorylase to phenylephrine (*left*) and epinephrine (*right*) in isolated rat hepatocytes. Three groups of animals were studied: age-matched controls (○), or 1–2-d NE-infused (●), and 4–7-d NE-infused (■) rats. Each point with a vertical bar represents the mean±SEM of at least five experiments. (*B*) Activation of glycogen phosphorylase to Arg-vasopressin, angiotensin-II, and the Ca^{2+} ionophore A23187 in isolated rat hepatocytes. Three groups of animals were studied: age-matched controls (○), 1–2-d NE-infused (●), and 4–7-d NE-infused (■) rats. Each point with a vertical bar represents the mean±SEM of five to seven experiments. (*C*) Activation of glycogen phosphorylase to glucagon, forskolin, and Bt_2cAMP in isolated rat hepatocytes. Three groups of animals were studied: age-matched controls (○), 1–2-d NE-infused (●), and 4–7-d NE-infused (■) rats. Each point with a vertical bar represents the mean±SEM of at least four experiments. *$P < 0.05$ difference from controls.

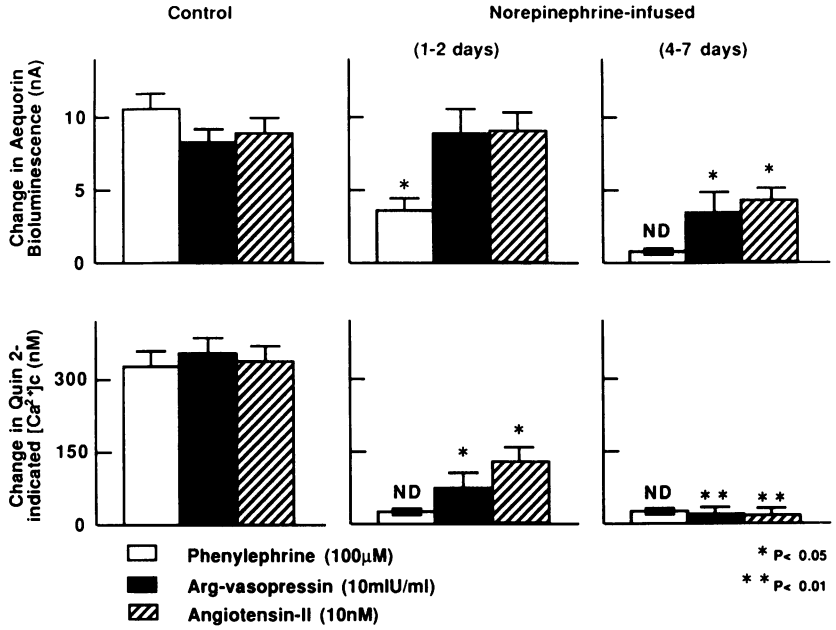

Figure 7. Effects of NE infusion on 100 μM phenylephrine-induced, 10 mIU/ml Arg-vasopressin-induced and 10 nM angiotensin II-induced changes in cytosolic free Ca^{2+} concentration in isolated rat hepatocytes. Three groups of animals were studied: age-matched controls (*left*), 1–2-d NE-infused (*middle*); or 4–7-d NE-infused (*left*) rats in medium containing 1.0 mM extracellular calcium. Values shown are the mean±SEM (*bars*) of five to six experiments. $*P < 0.05$ difference from controls; $**P < 0.01$ difference from controls.

gic receptor-mediated phosphorylase activation developed in the early stage of PHEO rats, downregulation of $alpha_1$ receptors and uncoupling at a step proximal to $alpha_1$ receptor-stimulated $[Ca^{2+}]c$ increase were found. On the other hand, heterologous desensitization seen in the later stage of the tumor development or the NE-infused rats also involved alteration(s) at steps distal to the rise in $[Ca^{2+}]c$.

Our study shows that the homologous desensitization of hepatic phosphorylase activation to $alpha_1$-adrenergic stimulation seen in the early PHEO rats involves a marked alteration in the relationship between $alpha_1$ receptor occupancy and receptor-coupled $[Ca^{2+}]c$ response. Under basal conditions, the relationship as defined by the phenoxybenzamine inactivation method was linear, indicating an absence of a receptor reserve in activating this intracellular event in isolated rat hepatocytes. This is in good agreement with previous observations by Lynch et al. (37) on the agonist concentration-response relationship in the same tissue; however, this situation seen in the liver is in sharp contrast to the nonlinearity between the same parameters in vascular smooth muscle cells, as shown by Colucci and Alexander (7). Based on the linear relationship in rat liver cells, the blunted $[Ca^{2+}]c$ response to $alpha_1$-adrenergic stimulation cannot be explained by the 34% decrease in $alpha_1$ receptor number that was actually detected. Therefore, uncoupling in receptor occupancy-$[Ca^{2+}]c$ response at a step proximal to $[Ca^{2+}]c$ mobilization and downregulation of receptors, are involved in the process of this type of desensitization. Furthermore, in the desensitization that developed in the NE-infused rats within 1–2 d, the phenylephrine-induced $[Ca^{2+}]c$ response was found to be significantly reduced without downregulation of $alpha_1$ receptors. This observation further supports the hypothesis that an alteration in receptor occupancy-$[Ca^{2+}]c$ response coupling, rather than downregulation, is of primary importance in the process of desensitization at a step proximal to $[Ca^{2+}]c$ mobilization.

The exact biochemical mechanism for this uncoupling of $alpha_1$ receptors is uncertain from this study, especially since our radioligand binding study could not detect any alteration in the binding properties of $alpha_1$ receptors for [^{3}H]prazosin in the desensitized hepatocytes. Using a different model system of smooth muscle cells, this functional uncoupling of $alpha_1$-adrenergic receptors to the PI turnover/$[Ca^{2+}]c$ mobilization pathway has been well studied (6, 7). We previously found that in vitro, 7 h exposure of rabbit aorta to $alpha_1$-adrenergic agonists leads to homologous desensitization of $alpha_1$-receptor–mediated smooth muscle contraction. This desensitization of contraction was not due to a change in $alpha_1$-adrenergic receptor number or affinity for agonists, but there was a blunting of NE-stimulated PI turnover (6). Similar results have been confirmed using cultured vascular smooth muscle cells from rabbit aorta (7). Furthermore, Leeb-Lundberg et al. recently found that functional uncoupling of $alpha_1$-adrenergic receptor-mediated PI metabolism in transformed smooth muscle cells (DDT_1 MF-2) is closely related with the phosphorylation of the M_r 80,000 $alpha_1$-adrenergic receptor ligand binding peptide, but the phosphorylation is without effect on its binding properties (38). Thus, the covalent modification of plasma membrane receptors by phosphorylation seems to play an important role in the desensitization of not only receptors that couple with adenylate cyclase but also those that couple to PI turnover/$[Ca^{2+}]c$ mobilization pathway. Further studies are clearly required to explain whether the possible phosphorylation of receptors by these mechanisms is causally related with the uncoupling seen in the in vivo homologous desensitization of hepatic phosphorylase responses.

To analyze the mechanism for the heterologous desensitization, it is important to determine first which $[Ca^{2+}]c$ signal being investigated better reflects the physiologically important $[Ca^{2+}]c$ change, since there was an apparent disparity between aequorin and quin2 results in the hepatocytes exerting heterologous type of desensitization. We have used both the fluorphore quin2 and the Ca^{2+}-sensitive photoprotein aequorin in this study, as these indicators have been reported to reflect different aspects of Ca^{2+} homeostasis; aequorin may be more sensitive to local and low levels of Ca^{2+} transients, whereas quin2 may respond more to the average or diffuse $[Ca^{2+}]c$ (39). Characterizing both aequorin- and quin2-indicated $[Ca^{2+}]c$

signals in rat hepatocytes, we found that they were generally similar in their nature, except for the time-course of the response. Aequorin-indicated response shows a very transient rise that returns to the baseline within 1–2 min, whereas the quin2-indicated one is more sustained and apparently well correlated with the changes in phosphorylase a. The similarity in the time course, however, does not necessarily mean that the quin2 response is better correlated with functionally important changes in $[Ca^{2+}]c$. For example, in platelets where the same situation exists for the time course of changes in $[Ca^{2+}]c$ and functional correlates, aequorin-indicated $[Ca^{2+}]c$ appears to be more closely related to both stimulatory and inhibitory changes in shape change, aggregation, or secretion than are rises in the quin2-indicated $[Ca^{2+}]c$ (40, 41). Thus, from the present characterization it is hard to determine which signal better reflects the $[Ca^{2+}]c$ change responsible for phosphorylase activation in hepatocytes.

With the difficulty in interpreting the $[Ca^{2+}]c$ results, heterologous desensitization seems to be a complex process and cannot be completely explained solely by alterations at a step proximal to $[Ca^{2+}]c$ mobilization. This type of desensitization of hepatic phosphorylase has been reported in several in vitro studies (13, 14). In one study, attenuation of agonist-promoted phosphorylase activity was observed to be paralleled by a decreased ability of the hormones to stimulate hydrolysis of inositol phospholipids, suggesting that the desensitization of glycogen phosphorylase activation involves unknown events that couple PI breakdown to intracellular Ca^{2+} mobilization (14). As shown in these in vitro studies and the homologous desensitization seen in the early stage of PHEO rats, regulation at the level of the receptor-$[Ca^{2+}]c$ response may represent one important group of adaptive responses directed at dampening excess stimulation, but these mechanisms do not exclude the role of modification of other cellular mechanisms in desensitization. Our data in the tolerant hepatocytes support the possibility that the in vivo development of this type of desensitization can be accomplished by a mechanism distal to $[Ca^{2+}]c$ mobilization as well, especially since phosphorylase activation by the Ca^{2+} ionophore A23187, which bypasses the receptor-mediated $[Ca^{2+}]c$ mobilization, is blunted in hepatocytes that exert heterologous type of desensitization.

The data indicate a near total loss of phosphorylase activation to all Ca^{2+}-linked stimulation after NE infusion or prolonged exposure to pheochromocytoma. Even in these tolerant hepatocytes cAMP-dependent phosphorylase activation by forskolin and Bt_2cAMP remains relatively unaffected, suggesting the defect is confined to Ca^{2+}-mobilizing pathway. However, the ability of glucagon in stimulating phosphorylase was found to be significantly decreased in these cells. These results indicate the site of the lesion for the reduced glucagon-stimulated response may lie at the level proximal to cAMP generation. Similar observations were also made in vitro (42), suggesting that specific desensitization of glucagon-stimulated adenylate cyclase can be elicited by hormones that stimulate the breakdown of inositol phospholipids and Ca^{2+} mobilization. Another important consideration with respect to the attenuated glucagon-stimulated phosphorylase activation in the tolerant hepatocytes is the possible existence of glucagon-stimulated PI turnover/Ca^{2+} mobilization pathway. The phosphorylase activation by glucagon is now suggested to be mediated by two distinct population of receptors, each couples with different second-messenger systems; one set is linked to stimulate PI turnover/Ca^{2+} mobilization pathway (GR1 receptors), and the other set being presumed to account for the stimulation of adenylate cyclase activity (GR2 receptors) (43). Consequently, it is possible that not only glucagon GR2 receptor-stimulated cAMP production system but also GR1 receptor-mediated PI/Ca^{2+} mobilization pathway itself is undergoing desensitization in these cells. These possibilities are now under investigation.

Desensitization was found in the ability of Ca^{2+}-mobilizing hormones to activate hepatic phosphorylase in both rats harboring PHEO and NE-infused rats. However, the picture and time course of desensitization was markedly different between these two models. The explanation for the differences between two animal models is unclear. Possible factors that could relate to this disparity involve the different NE delivery rate by the tumor from that by osmotic minipump; thus, plasma NE in the PHEO rats was reported to rise gradually to maximum levels by 4 wk after tumor transplantation (44), whereas plasma NE levels were elevated ~ 30-fold by 14 h, the earliest time tested, and remained at this level for all 7 d in the rats implanted with osmotic minipumps. Also, the potentially important role of the markedly elevated dopamine concentration in the tumor-bearing rats should be considered. Dopamine has been known to be a partial agonist at alpha-adrenergic receptors (45). Partial agonists may antagonize the effects of the endogenous full agonist, NE. Moreover, recent studies have revealed that PHEO may produce and secrete various neuropeptide-like substances besides catecholamines (46). Thus, this multisecretory nature of this amine-precursor uptake decarboxylation tumor could contribute the disparity observed. Whatever the explanation, the current results emphasize that one must be careful in extrapolating findings obtained for desensitization of adrenergic receptor functions in the PHEO model to other settings. The similar observation that increases in catecholamines by implantation of PHEO or infusion of agonists produce somewhat different patterns of regulation of adrenergic receptors has been documented previously in rat renal cortex (47).

In conclusion, our results indicate the complexity and diversity of mechanisms that may mediate desensitization to excessive in vivo stimulation of Ca^{2+}-messenger system. Not only may desensitization occur through functional uncoupling and downregulation of receptors but also postreceptor mechanism(s), including distal to $[Ca^{2+}]c$ mobilization may be involved in certain cells. Hepatocytes may be a useful model system to pursue further these more poorly understood possibilities.

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References

1. Lefkowitz, R. J., J. M. Stadel, and M. G. Caron. 1983. Adenylate cyclase coupled beta-adrenergic receptors: structure and mechanisms of activation and desensitization. *Annu. Rev. Biochem.* 52:159–186.

2. Harden, T. K. 1983. Agonist-induced desensitization of the beta-adrenergic receptor-linked adenylate cyclase. *Pharmacol. Rev.* 35:5–32.

3. Exton, J. H. 1986. Mechanisms involved in calcium-mobilizing agonist responses. *In* Advances in Cyclic Nucleotide and Protein Phosphorylation Research. Vol. 20. P. Greengard and G. A. Robison, editors. Raven Press, New York. 211–262.

4. Rasmussen, H. 1986. The calcium messenger system. *N. Engl. J. Med.* 314:1094–1101 (Pt. 1), 314:1164–1170 (Pt. 2).

5. Hoffman, B. B., and R. J. Lefkowitz. 1980. Alpha-adrenergic receptor subtypes. *N. Engl. J. Med.* 302:1390–1396.

6. Lurie, K. G., G. Tsujimoto, and B. B. Hoffman. 1985. Desensitization of alpha$_1$ adrenergic receptor-mediated vascular smooth muscle contraction. *J. Pharmacol. Exp. Ther.* 234:147–152.

7. Colucci, W. S., and R. W. Alexander. 1986. Norepinephrine-induced alteration in the coupling of α_1-adrenergic receptor occupancy to calcium efflux in rabbit aortic smooth muscle cells. *Proc. Natl. Acad. Sci. USA.* 83:1743–1746.

8. Pollock, W. K., and D. E. MacIntyre. 1986. Desensitization and antagonism of vasopressin-induced phosphoinositide metabolism and elevation of cytosolic free calcium concentration in human platelets. *Biochem. J.* 234:67–73.

9. De Togni, P., V. Della Bianca, M. Grzeskowiak, F. Di Virgillo, and F. Rossi. 1985. Mechanism of desensitization of neutrophil response to N-formylmethionylleucylphenylalanine by slow rate of receptor occupancy. Studies on changes in Ca^{2+} concentration and phosphatidylinositol turnover. *Biochim. Biophys. Acta.* 838:23–31.

10. Exton, J. H. 1985. Mechanisms involved in α-adrenergic phenomena. *Am. J. Physiol.* 248:E633–647.

11. Williamson, J. R., R. H. Cooper, S. K. Joseph, and A. P. Thomas. 1985. Inositol triphosphate and diacylglycerol as intracellular second messengers in liver. *Am. J. Physiol.* 248:C203–C216.

12. Chrisman, T. D., J. E. Jordan, and J. H. Exton. 1982. Purification of rat liver phosphorylase kinase. *J. Biol. Chem.* 257:10798–10804.

13. Breant, B., S. Keppens, and H. De Wulf. 1981. Heterologous desensitization of the cyclic AMP-independent glycogenolytic response in rat liver cells. *Biochem. J.* 200:509–514.

14. Kirk, C. J., and J. A. Creba. 1982. Heterologous desensitization of phosphorylase activation and phosphatidylinositol 4,5-biphosphate breakdown provoked by Ca^{2+}-mobilizing hormones in hepatocytes. *Biochem. Soc. Trans.* 10:264–265.

15. Warren, S., and R. N. Chute. 1972. Pheochromocytoma. *Cancer (Phila.).* 29:327–331.

16. Tsujimoto, G., W. M. Manger, and B. B. Hoffman. 1984. Desensitization of β-adrenergic receptors by pheochromocytoma. *Endocrinology.* 114:1272–1278.

17. Tsujimoto, G., K. Honda, B. B. Hoffman, and K. Hashimoto. 1987. Desensitization of postjunctional α_1- and α_2-adrenergic receptor-mediated vasopressor responses in rat harboring pheochromocytoma. *Circ. Res.* 61:86–98.

18. Mefford, I. N., M. M. Ward, L. Miles, B. Tailor, M. A. Chesney, D. L. Keegan, and J. D. Barchas. 1981. Determination of plasma catecholamines and free 3,4-dihydroxyphenyl acetic acid in continuously collected human plasma by high performance liquid chromatography with electrochemical detection. *Life Sci.* 28:477–483.

19. Ohnishi, A., A. Minegishi, T. Sakaki, T. Suganuma, and T. Ishizaki. 1987. Effect of β-adrenoceptor blockade on exercise-induced plasma catecholamine concentrations and their dissipation profile. *Br. J. Clin. Pharmacol.* 23:339–343.

20. Bissell, D. M., and P. S. Guzelian. 1980. Phenotypic stability of adult rat hepatocytes in primary monolayer culture. *Ann. NY Acad. Sci.* 349:85–98.

21. Berry, M. N., and D. S. Friend. 1969. High-yield preparation of isolated rat liver parenchymal cells. *J. Cell Biol.* 43:506–520.

22. Tsujimoto, A., G. Tsujimoto, K. Kato, and K. Hashimoto. 1986. Developmental alteration in adrenergic regulation of hepatic glycogen phosphorylase. *Jpn. J. Pharmacol.* 40:161–168.

23. Besse, J. C., and R. F. Furchgott. 1976. Dissociation constants and relative efficacies of agonists acting on alpha adrenergic receptors in rabbit aorta. *J. Pharmacol. Exp. Ther.* 197:66–78.

24. Mauger, J.-P., F. Sladeczek, and J. Bockaert. 1982. Characteristics and metabolism of α_1 adrenergic receptors in a nonfusing muscle cell line. *J. Biol. Chem.* 257:875–879.

25. Gilboe, D. P., K. L. Larson, and F. Q. Nuttall. 1972. Radioactive method for the assay of glycogen phosphorylases. *Anal. Biochem.* 47:20–27.

26. Tsujimoto, A., G. Tsujimoto, S. Azhar, and B. B. Hoffman. 1986. Altered responsiveness to alpha- and beta-adrenoceptor stimulation in hepatocytes cultured in defined medium. *Biochem. Pharmacol.* 35:1400–1404.

27. Borle, A. B., C. C. Freudenrich, and K. W. Snowdowne. 1986. A simple method for incorporating aequorin into mammalian cells. *Am. J. Physiol.* 251:C323–C326.

28. Borle, A. B., and K. W. Snowdowne. 1985. Measurement of intracellular ionized calcium with aequorin. *Methods Enzymol.* 124:90–116.

29. Snowdowne, K. W., and A. B. Borle. 1984. Measurement of cytosolic free calcium in mammalian cells with aequorin. *Am. J. Physiol.* 247:C396–C408.

30. Thomas, A. P., J. Alexander, and J. R. Williamson. 1984. Relationship between inositol polyphosphate production and the increase of cytosolic free Ca^{2+} induced by vasopressin in isolated hepatocytes. *J. Biol. Chem.* 259:5574–5584.

31. Tsien, R. Y., T. Pozzan, and T. J. Rink. 1982. Calcium homeostasis in intact lymphocytes: cytoplasmic free calcium monitored with a new intracellularly trapped fluorescent indicator. *J. Cell Biol.* 94:325–334.

32. Tsujimoto, A., G. Tsujimoto, and B. B. Hoffman. 1986. Age-related changes in adrenergic regulation of glycogen phosphorylase in rat hepatocytes. *Mech. Ageing Dev.* 33:167–175.

33. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265–275.

34. DeLean, A., P. J. Munson, and D. Rodbard. 1978. Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curves. *Am. J. Physiol.* 235:E97–E102.

35. Munson, P. J., and D. Rodbard. 1980. LIGAND: a versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.* 107:220–239.

36. Su, Y. F., L. Cubeddu, and J. P. Perkins. 1976. Regulation of adenosine 3′:5′-monophosphate content of human astrocytoma cells: desensitization to catecholamines and prostaglandins. *J. Cyclic Nucleotide Res.* 2:257–270.

37. Lynch, C. J., P. F. Blackmore, R. Charest, and J. H. Exton. 1985. The relationships between receptor binding capacity for norepinephrine, angiotensin II, and vasopressin and release of inositol trisphosphate, Ca^{2+} mobilization, and phosphrylase activation in rat liver. *Mol. Pharmacol.* 28:93–99.

38. Leeb-Lundberg, L. M. F., S. Cottechia, A. DeBlasi, M. G. Caron, and R. J. Lefkowitz. 1987. Regulation of adrenergic receptor function by phosphorylation I: agonist-promoted desensitization and phosphorylation of α_1-adrenergic receptors coupled to inositol phospholipid metabolism in DDT$_1$ MF-2 smooth muscle cells. *J. Biol. Chem.* 262:3098–3105.

39. Johnson, P. C., J. A. Ware, P. B. Cliveden, M. Smith, A. M.

Dvorak, and E. W. Salzman. 1985. Measurement of ionized calcium in blood platelets with the photoprotein aequorin: comparison with quin 2. *J. Biol. Chem.* 260:2069–2076.

40. Ware, J. A., P. C. Johnson, M. Smith, and E. W. Salzman. 1986. Effect of common agonists on cytoplasmic ionized calcium concentration in platelets. *J. Clin. Invest.* 77:878–886.

41. Ware, J. A., P. C. Johnson, M. Smith, and E. W. Salzman. 1986. Inhibition of human platelet aggregation and cytoplasmic calcium response by calcium antagonists: studies with aequorin and quin2. *Circ. Res.* 59:39–42.

42. Murphy, G. J., V. J. Hruby, D. Trivedi, M. J. O. Wakelam, and M. D. Houslay. 1987. The rapid desensitization of glucagon-stimulated adenylate cyclase in a cyclic AMP-independent process that can be mimicked by hormones which stimulate inositol phospholipid metabolism. *Biochem. J.* 243:39–46.

43. Wakelam, M. J. O., G. J. Murphy, V. J. Hruby, and M. D. Houslay. 1986. Activation of two signal-transduction systems in hepatocytes by glucagon. *Nature (Lond.).* 323:68–71.

44. Alexander, N., S. Yoneda, and N. D. Vlachakis. 1985. Catecholamines in plasma and erythrocytes of rats with transplantable pheochromocytoma. *Life Sci.* 36:571–577.

45. Goldberg, L. I., J. D. Kohli, A. N. Kotake, and P. H. Volkman. 1978. Characteristics of the vascular dopamine receptor: comparison with other receptors. *Fed. Proc.* 37:2396–2402.

46. Hassoun, J., G. Monges, P. Giraud, J. F. Henry, C. Charpin, H. Payan, and M. Toga. 1984. Immunohistochemical study of pheochromocytomas: an investigation of methionine-enkephalin, vasoactive intestinal peptide, somatostatin, corticotropin, β-endorphin, and calcitonin in 16 tumors. *Am. J. Pathol.* 114:56–63.

47. Snavely, M. D., M. G. Ziegler, and P. A. Insel. 1985. Subtype-selective down-regulation of rat renal cortical α- and β-adrenergic receptors by catecholamines. *Endocrinology.* 117:2182–2189.