0026-895X/87/010043-10$02.00/0

MOLECULAR PHARMACOLOGY, 32:43-52

The Relationship Between α_1-Adrenergic Receptor Occupancy and Response in BC3H-1 Muscle Cells

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Received January 13, 1987; Accepted April 14, 1987

SUMMARY

The relationship between α_1-adrenergic receptor occupancy by agonists or antagonists and the regulation of intracellular Ca^{2+} was examined. Receptor occupancy was measured using the antagonist [^{3}H]prazosin and correlated with agonist-elicited $^{45}Ca^{2+}$ fluxes. The agonists epinephrine (E), norepinephrine (NE), and phenylephrine (PE) coordinately activated Ca^{2+} efflux, reflecting a substantial mobilization of intracellular Ca^{2+}, as well as a smaller $^{45}Ca^{2+}$ influx. The agonist concentration dependences for influx and efflux were similar, with the order of potency expected for α_1 receptors (E $\geq$ NE > PE). To determine the relationship between receptor occupancy and response, the slowly dissociating antagonist prazosin was used to inactivate specified fractions of the receptor population. A linear relationship was observed between the remaining activatable receptors and residual $^{45}Ca^{2+}$ efflux elicited by E or NE, except at saturating agonist concentrations where some curvature was observed. Moreover, the concentration dependence for agonist-elicited $^{45}Ca^{2+}$ efflux was shifted toward slightly higher concentrations of E or NE following prazosin inactivation. These results suggest the presence of a modest receptor reserve which is revealed by E or NE, but not by PE. Agonist occupation was measured over the same interval as receptor activation by competition with the initial rate of [^{3}H]prazosin association. All three agonists exhibited the major fraction of receptor occupation over the same concentration ranges required for the functional response. Exposure of receptors to specified agonist concentrations for 30 min had little effect on the number of receptors or their ligand affinities, whereas a 2.5-hr exposure to agonist decreased apparent agonist affinity as well as the number of receptors recognized by [^{3}H]prazosin.

Agonist occupation of cell surface α_1-adrenergic receptors in vascular smooth muscle initiates a sequence of events leading to elevation of intracellular free Ca^{2+} and consequent activation of muscle contraction. In theory, receptor activation could deliver Ca^{2+} to the contractile apparatus either by mobilizing Ca^{2+} from intracellular organelles or by elevating transmembrane Ca^{2+} entry, allowing Ca^{2+} to move down its inwardly directed concentration gradient. Both mechanisms have been documented experimentally, and their relative contributions vary depending on the tissue source and mode of stimulation (1). Quantitative measurements of the relationship between receptor occupancy and cellular responses have provided additional insight into the signal transduction mechanism (2).

An important feature of pharmacological receptors is that their efficiency of coupling to cellular responses may be influenced by the past history of receptor activity. Thus, previous intervals of agonist exposure rapidly convert β-adrenergic (3) and nicotinic acetylcholine receptors (4, 5) to states which exhibit altered agonist binding properties and a decrement in the ability of agonist to elicit a functional response. In the case of receptors which regulate intracellular Ca^{2+}, prolonged agonist stimulation may lead to hysteresis so that cellular responses are maintained during prolonged agonist stimulation, yet steady state concentrations of Ca^{2+} are only slightly elevated from resting values (6).

The BC3H-1 cell line has been developed as a simplified model for studying questions relating to α_1 receptor regulation of intracellular Ca^{2+}. Previous studies have shown that activation of α_1-adrenergic receptors on these cells by the agonist phenylephrine predominantly mobilizes Ca^{2+} from intracellular stores, which can be monitored as $^{45}Ca^{2+}$ unidirectional efflux and increased intracellular free Ca^{2+} concentrations. A smaller increase in transmembrane Ca^{2+} entry is also detected upon receptor activation (7, 8). A linear relationship was observed between the number of activatable receptors which remained after inactivation by either phenoxybenzamine or the slowly dissociating antagonist prazosin relative to the residual $^{45}Ca^{2+}$ efflux response to phenylephrine, indicating the absence of appreciable receptor reserves (9). More recently, we have examined the influence of prior agonist exposure on receptor responsiveness and have found that Ca^{2+} efflux in response to

This work was supported by Grant-in-Aid 84-S105 from the American Heart Association, California Affiliate (to R. D. B.) and National Institutes of Health Grant HL-25457 (to P. T.).

ABBREVIATIONS: HEPES, 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid; quin 2, 2-[[2-bis-(carboxymethyl)-amino-5-methyl phenoxy]-methyl]-6-methoxy-8-bis-(carboxymethyl)-amino-quinoline.

the agonist norepinephrine is maintained substantially intact during a 30-min interval of agonist exposure (10).

These studies have now been extended to the catecholamine agonists epinephrine and norepinephrine. The adrenergic agonists coordinately activate the mobilization of intracellular Ca^{2+} as well as transmembrane Ca^{2+} entry. Receptor inactivation experiments suggest the presence of a modest receptor reserve which is detected by epinephrine and norepinephrine, in contrast to the previous results with phenylephrine. In addition, we have developed a means of ascertaining agonist occupation within the brief time interval in which the functional responses are measured. This procedure allows estimation of agonist occupation prior to occurrence of state changes resulting from prolonged agonist exposure. By independently assessing agonist occupancy from competition with initial rates of [^{3}H]prazosin association, it has been possible to examine agonist occupation and receptor numbers prior to and following prolonged exposure to agonist. This has permitted us to compare agonist occupation directly with the functional response and to examine the influence of prolonged agonist exposure on properties of receptor occupancy. A preliminary account of this work has already appeared (11).

Experimental Procedures

Materials

l-Epinephrine *d*-bitartrate, *l*-norepinephrine *d*-bitartrate, *l*-phenylephrine hydrochloride, superoxide dismutase, and catalase were obtained from Sigma. Catecholamine stock solutions were prepared in 1 mM HCl. Catecholamine oxidation in physiological buffers was avoided by addition of superoxide dismutase and catalase, each at 10 μg/ml (12). Phentolamine hydrochloride was a gift from Dr. Richard Hughes in the Division of Pharmacology. Prazosin hydrochloride was a gift from Pfizer (Groton, CT). [^{3}H]Prazosin (82 Ci/mmol) was obtained from New England Nuclear and $^{45}Ca^{2+}$ was obtained from Amersham. Cell culture media were obtained from Gibco and fetal bovine serum was obtained from Irvine Scientific Co. (Irvine, CA). The BC3H-1 cell line was propagated and growth of experimental cultures was performed as described previously (7).

Measurement of Unidirectional $^{45}Ca^{2+}$ Fluxes

Procedures for equilibration of monolayer BC3H-1 cultures with tracer $^{45}Ca^{2+}$ and measurement of radioisotope efflux from the cells were described previously (7, 10). Unidirectional $^{45}Ca^{2+}$ influx was also measured as described (10).

Occupation and Inhibition of α_1 Receptors by Prazosin

Measurements of equilibrium receptor occupation by [^{3}H]prazosin or nonradioactive prazosin and inhibition of unidirectional $^{45}Ca^{2+}$ efflux were performed as described previously (9).

Agonist Competition with Initial Rate of [^{3}H]prazosin Association

Measurements of agonist occupation by competition with the initial rate of [^{3}H]prazosin association were performed by the following three-step sequence of a prior agonist conditioning interval, a [^{3}H]prazosin association interval, and a nonspecific [^{3}H]prazosin dissociation interval.

a) Agonist conditioning interval. Each culture was washed sequentially with two 3-ml aliquots of physiological buffer (composition in mM: NaCl, 140; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 1.0; D-glucose, 10.0; HEPES, 25, pH 7.4) to remove experimental growth medium, and a third aliquot of buffer containing the specified agonist concentration was applied to the culture for indicated time intervals on a 37° water bath.

b) [^{3}H]Prazosin association interval. The agonist conditioning solution from step a was aspirated and the kinetic assay was immediately initiated by applying a fresh solution of buffer containing 3 nM [^{3}H]prazosin plus appropriate concentrations of competing ligands. Nonspecific binding was defined by measuring [^{3}H]prazosin association in the presence of 10 μM phentolamine. Association proceeded for 20 sec at 37°. The reaction was terminated by aspirating the assay solution and sequentially washing the culture with four 3-ml aliquots of buffer containing 10 μM phentolamine at 37°.

c) Nonspecific [^{3}H]prazosin dissociation interval. The fourth aliquot of phentolamine-supplemented buffer from step b was incubated with the culture for 15 min on the 37° bath. Following this interval, the solution was aspirated, and the monolayer was detergent solubilized and processed for liquid scintillation counting of bound [^{3}H] prazosin in the usual manner.

Kinetic data for [^{3}H]prazosin association were corrected for nonspecific binding and analyzed as a pseudo-first order approach to equilibrium (9). The number of prazosin-binding sites at equilibrium was measured independently in the same set of experimental cultures using 500 pM [^{3}H]prazosin with nonspecific binding defined using 10 μM phentolamine. Rate constants obtained in the presence of competing ligand (k_p) were expressed as a fraction of the control rate of [^{3}H] prazosin association (k_p^{max}) measured in the absence of competing ligand.

Data Analysis

Experimental determinations were routinely performed on triplicate BC3H-1 cultures, and the data shown represent mean values compiled from replicate experiments. The number of replicate experiments, *n*, accompanies each figure legend. Estimates of numerical constants were obtained from the experimental data using nonlinear regression methods implemented with the GraphPAD program developed by Dr. Harvey Motulsky in the Division of Pharmacology. Additional details are provided in the text.

Results

Concentration dependence for agonist-elicited unidirectional $^{45}Ca^{2+}$ efflux. Previous studies with BC3H-1 cells have established that unidirectional $^{45}Ca^{2+}$ efflux elicited by agonists reflects the mobilization of sequestered intracellular Ca^{2+} (7). This measurement may be employed as a simple assay of receptor function. Fig. 1 shows the concentration dependences for $^{45}Ca^{2+}$ efflux in response to epinephrine (E), norepinephrine (NE), and phenylephrine (PE). The observed order of agonist potency (E ≥ NE > PE) is consistent with the prediction for an α_1 receptor-mediated response. In addition, small differences were observed in the magnitudes of $^{45}Ca^{2+}$ efflux responses to maximally effective concentrations of the three agonists. These differences again paralleled the expected potency order for α_1 receptors (E ≥ NE > PE). The diminished maximal response of phenylephrine indicated that it behaves as a partial agonist in this system. Parameters for agonist activation of $^{45}Ca^{2+}$ efflux are compiled in Table 1. We ruled out the possibility that these responses were modified by contributions from α_2 or β-adrenergic receptor activation. BC3H-1 cells lack detectable α_2-adrenergic receptors. All of the agonist-elicited responses are completely inhibited by the α_1 antagonist prazosin over the appropriate concentration range (data not shown). Neither the β-adrenergic agonist isoproterenol (10^{-7} M) nor the β-adrenergic antagonist propranolol (10^{-6} M) had any effect on basal or agonist-stimulated $^{45}Ca^{2+}$ efflux (data not shown).

Agonist activation of unidirectional $^{45}Ca^{2+}$ influx. The ability of maximally effective concentrations of the adrenergic

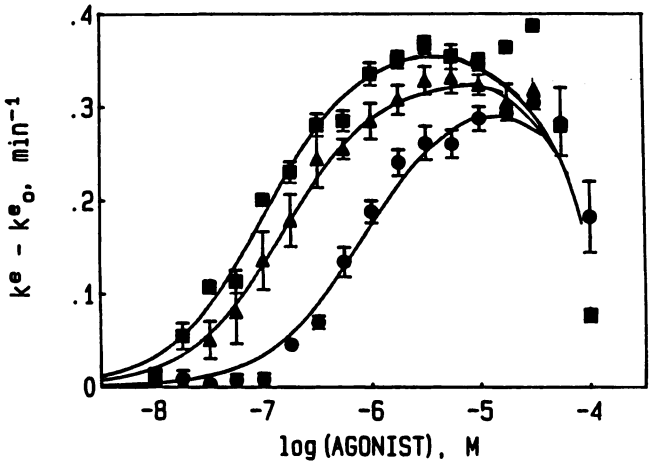

Fig. 1. Concentration dependence for agonist-elicited unidirectional $^{45}Ca^{2+}$ efflux in BC3H-1 cells. Unidirectional $^{45}Ca^{2+}$ efflux was measured over a 3-min interval in the presence of the indicated agonist concentrations following a 10-min incubation in buffer to remove rapidly exchangeable cellular $^{45}Ca^{2+}$ as described under Experimental Procedures. Efflux data were treated as a first order process. Experimentally determined rate constants (k^e) were corrected for basal efflux (k_o^e). ■, epinephrine; ▲, norepinephrine; ●, phenylephrine. Data represent means ± standard errors compiled from four to seven separate experiments.

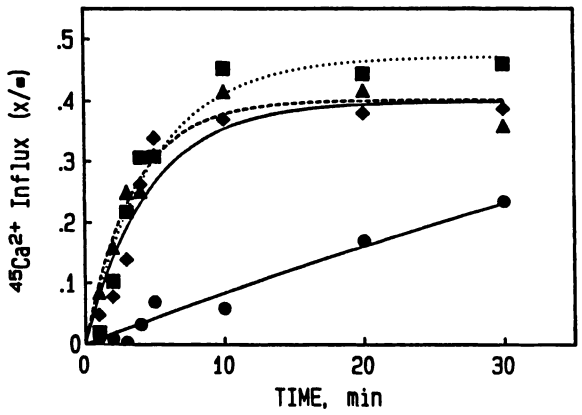

Fig. 2. Kinetics of agonist-elicited unidirectional $^{45}Ca^{2+}$ influx. Unidirectional $^{45}Ca^{2+}$ influx was measured by a modification of our previous procedure (10). Cultures were equilibrated for 10 min on a 37° water bath in 1.0 ml of buffer. Influx was initiated by adding a further 1.0-ml aliquot of physiological buffer (37°) containing $^{45}Ca^{2+}$ and appropriate agonists at twice their desired final concentrations with gentle mixing. Influx was terminated at the appropriate time interval by washing the monolayer with four 3-ml aliquots of ice-cold buffer containing 5 mM $LaCl_3$. Data were corrected for rapidly exchanging and nonspecific $^{45}Ca^{2+}$ uptake and were expressed as a first order exponential approach to radioisotopic equilibrium as described in Experimental Procedures (n = 3). ◆, epinephrine, 2 μM; ▲, norepinephrine, 2 μM; ■, phenylephrine, 6 μM; ●, no added agonist.

agonists to elicit $^{45}Ca^{2+}$ influx was measured as shown in Fig. 2. Data were analyzed as a first order approach to radioisotopic equilibrium. The kinetics of unidirectional $^{45}Ca^{2+}$ influx were fit according to Eq. 1 (14).

$$\frac{x}{\infty} = A(1 - e^{-k_i t}) \qquad (1)$$

Here ∞ represents exchangeable cellular $^{45}Ca^{2+}$ at radioisotopic equilibrium, x equals cellular $^{45}Ca^{2+}$ uptake at time t, A represents the agonist-sensitive fraction of exchangeable cellular Ca^{2+}, and k_i is the rate constant for $^{45}Ca^{2+}$ exchange. Estimated kinetic parameters appear in Table 1. Precise quantitation of agonist-elicited $^{45}Ca^{2+}$ influx is precluded by the small magnitude of the response relative to unstimulated $^{45}Ca^{2+}$ influx. The kinetic analysis is further complicated since the size of the intracellular compartment decreases during the interval of the influx measurement. This reduction in total intracellular Ca^{2+} is reflected in a decline in exchangeable cellular $^{45}Ca^{2+}$ at radioisotopic equilibrium and a reduction in total cellular Ca^{2+} content determined by atomic absorption spectroscopy (7). Unidirectional influx most accurately approximates first order kinetics during the early time points, and with Eq. 1 we estimate the initial phase of the kinetic course. Similar responses were obtained to maximally effective concentrations of each agonist.

In additional experiments the concentration dependence for agonist-elicited $^{45}Ca^{2+}$ influx obeyed the same rank order of potency as previously described for unidirectional $^{45}Ca^{2+}$ efflux (data not shown). Thus, adrenergic agonists concomitantly activate $^{45}Ca^{2+}$ influx as well as efflux, but the influx is quantitatively of smaller magnitude and hence total intracellular Ca^{2+} is depleted.

The concentration dependence for agonist-elicited $^{45}Ca^{2+}$ efflux following inactivation of receptors by prazosin. The rate constant for prazosin dissociation from the α_1 receptor (0.018 min^{-1}, $t_{1/2}$ = 38 min) is slow relative to the 3-min interval employed in the unidirectional $^{45}Ca^{2+}$ efflux assay (9). Consequently, prior occupation of α_1 receptors by prazosin pseudo-irreversibly and, thus, noncompetitively inhibits agonist-activated $^{45}Ca^{2+}$ efflux. When increasing fractions of the receptor population are inactivated by prazosin, the concentration dependence for epinephrine activation shifts toward higher epinephrine concentrations in addition to the expected decrease in the maximum obtainable response as shown in Fig. 3A. The magnitude of the concentration shift is not large; only a 2- to 5-fold increase was observed over a prazosin concentration range which inhibited 70% of the agonist-elicited efflux. This result suggests the presence of a modest receptor reserve for

TABLE 1

Activation parameters for α_1-adrenergic agonist-elicited $^{45}Ca^{2+}$ flux

Unidirectional $^{45}Ca^{2+}$ efflux data in Fig. 1 were analyzed by the law of mass action to determine k_g^{max}, the maximum agonist-elicited response, and EC_{50}, the agonist concentration which produced a half-maximal response. $^{45}Ca^{2+}$ efflux was treated as a first order process over the assay interval (9, 13). Unidirectional $^{45}Ca^{2+}$ influx data in Fig. 2 were analyzed according to Eq. 1 to determine, k_i, the initial rate of agonist-elicited $^{45}Ca^{2+}$ influx, and A, the fraction of the agonist-sensitive $^{45}Ca^{2+}$ influx component relative to total exchangeable cellular $^{45}Ca^{2+}$.

Agonist	$^{45}Ca^{2+}$ efflux		$^{45}Ca^{2+}$ influx	
	EC_{50}	k_g^{max}	A	k_i
	M	min^{-1}		min^{-1}
Phenylephrine	8.02 ± 0.92 × 10^{-7}	0.31 ± 0.01	0.47 ± 0.03	0.205 ± 0.04
Norepinephrine	1.53 ± 0.15 × 10^{-7}	0.33 ± 0.01	0.40 ± 0.02	0.283 ± 0.04
Epinephrine	1.03 ± 0.09 × 10^{-7}	0.37 ± 0.01	0.40 ± 0.03	0.217 ± 0.05

epinephrine activation. Small shifts were also observed for the concentration dependence of norepinephrine activation following prazosin occupation (Fig. 3B). Previous studies with phenylephrine showed that the maximum response decreased following prazosin occupation without altering the concentration dependence for phenylephrine activation, arguing against the existence of a receptor reserve for this agonist (9). The magnitude of the shift in the concentration dependence for agonist activation following receptor inactivation has been previously employed to calculate K_A, the agonist dissociation constant, according to Eq. 2 (2).

$$\frac{1}{A} = \frac{1}{q} \cdot \frac{1}{A'} + \frac{(1-q)}{q \cdot K_A} \qquad (2)$$

Here A and A' are agonist concentrations which produce equivalent functional responses in the presence and absence of receptor inactivation, respectively. A plot of $1/A$ versus $1/A'$ allows calculation of q, the residual fraction of active receptors following prazosin occupation, and K_A, the agonist dissociation constant. This analysis was performed on replicate experiments represented by the data in Fig. 3, and the results appear in Table 2. The K_A value calculated for epinephrine was $2.87 \pm 0.69 \times 10^{-7}$ M and that for norepinephrine was 2.86×10^{-7} M. From these values of K_A and EC_{50}, the fractional receptor occupancies necessary to elicit 10%, 50%, and 90% responses were calculated for both agonists from the law of mass action. For epinephrine, 10% response required 4% occupancy, 50% response required 26% occupancy, and 90% response required 76% occupancy. For norepinephrine, 10% response required 6% occupancy, 50% response required 35% occupancy, and 90% response required 83% occupancy. These calculations confirm that only small receptor reserves exist and suggest that the two catecholamines activate the receptor with similar efficacies.

[^{3}H]Prazosin occupancy and inhibition of agonist-elicited $^{45}Ca^{2+}$ efflux. The preceding experiment detected the presence of a small receptor reserve for the catecholamine agonists by measuring the concentration dependence for agonist activation following prazosin occupation. This result has been confirmed by direct measurement of receptor occupancy by specified concentrations of [^{3}H]prazosin combined with parallel measurement of the residual functional response to a test agonist concentration. Fig. 4 shows the relationship between the number of activatable receptors which remain following occupation with [^{3}H]prazosin and the residual functional response to epinephrine (Fig. 4A) or norepinephrine (Fig. 4B). At a subsaturating concentration (1 μM) of either agonist, a negative linear relationship is obtained between [^{3}H]prazosin occupancy and inhibition of functional response. The data obtained at 1 μM epinephrine correspond to a line of slope -0.95 ($r = -0.97$). For norepinephrine the slope is -0.93 ($r = -0.94$). This result indicates the absence of a receptor reserve under these conditions and is consistent with our previous experiments using phenylephrine (9). When saturating epinephrine concentrations were approached, we observed a convex, parabolic relation suggestive of a modest receptor reserve. This behavior is illustrated by the data obtained at 6 μM epinephrine in Fig. 4A. Using the formalism developed by Furchgott for a receptor reserve, we previously derived an expression to describe the shape of this plot as shown in Eq. 3 (9).

$$\frac{k'}{k} = \frac{q[1 + (A/K_A)(1+e)]}{[1 + (A/K_A)(1+qe)]} \qquad (3)$$

where k'/k is the fractional response remaining for agonist concentration A following receptor inactivation relative to the control case, e represents agonist efficacy, and the other terms retain their previous definitions. The magnitude of curvature of the plot is proportional to the extent of receptor reserve (measured as agonist efficacy) and the concentration of agonist relative to its dissociation constant. Thus, curvature should increase as maximally effective agonist concentrations are approached. The linear relationships obtained at subsaturating agonist would define a lower limit for agonist efficacy of 1.0. The data obtained at 6 μM epinephrine in Fig. 4A were fit to Eq. 3 in order to estimate an upper limit for epinephrine efficacy of 2.96 ± 0.81. Taken together, these data confirm the lack of substantial receptor reserves in this system.

Thus, the three agonists share a common ability to coordinately activate both Ca^{2+} entry and intracellular Ca^{2+} mobilization, although quantitative differences exist in the efficiency with which agonist occupation is coupled to the functional response.

Kinetics of [^{3}H]prazosin association and dissociation. The functional studies described to this point only provide an indirect measure of agonist occupancy of the receptor. The availability of [^{3}H]prazosin as a specific radioligand for the α_1 receptor allows direct measurement of agonist occupancy parameters by competition binding methods. However, exposure of receptors to agonist over the long time intervals needed to achieve equilibrium binding may result in state changes of the receptor which are reflected in altered agonist affinity. To circumvent this problem, we have devised a means to measure agonist affinity by competition with the initial rate of [^{3}H] prazosin association over the same time interval in which the

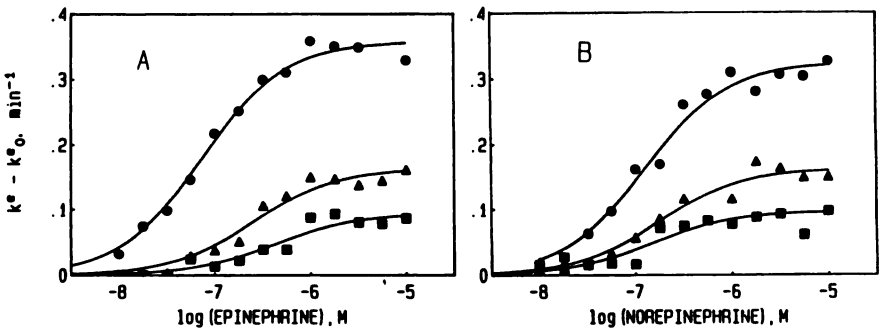

Fig. 3. Concentration dependence for agonist activation following prazosin occupation. Sets of cultures equilibrated overnight with $^{45}Ca^{2+}$ were incubated with specified prazosin concentrations during the final 2.5 hr of radioisotopic equilibration. Cultures were washed with buffer and rapidly exchanging $^{45}Ca^{2+}$ was removed during a 10-min incubation at 37°. A fresh aliquot of buffer containing agonist was applied and unidirectional $^{45}Ca^{2+}$ efflux was measured over a 3-min interval. A shows epinephrine activation and B shows norepinephrine activation following occupation by 56 pM prazosin (▲), 100 pM prazosin (■), or no added prazosin (●). Data are shown from representative experiments for each agonist; analyses of replicate experiments appear in Table 2.

TABLE 2

Parameters for agonist activation following prazosin occupation

The data from replicate experiments similar to those in Fig. 3 were analyzed according to the method of Furchgott (2) as described in the text in order to determine K_A, the agonist-receptor dissociation constant, and q, the fraction of activatable receptors remaining after prazosin inactivation.

Experiment	Agonist	Prazosin	q	K_A
		pM		M
1	Epinephrine	50	0.48	2.42×10^{-7}
2	Epinephrine	56	0.12	3.66×10^{-7}
3	Epinephrine	56	0.37	2.52×10^{-7}
4	Norepinephrine	56	0.29	4.39×10^{-7}
5	Norepinephrine	50	0.72	1.32×10^{-7}

functional response is being measured (5). Preliminary attempts to measure agonist competition with the initial rate of [^{3}H]prazosin association were unsuccessful because of the high levels of nonspecific binding encountered at the elevated prazosin concentrations used in the assay. The contribution of nonspecific binding was minimized by exploiting the observation that [^{3}H]prazosin dissociation from the α_1 receptor is slow relative to the dissociation of nonspecifically adsorbed [^{3}H]prazosin from the intact cell (9). The utility of this strategy is illustrated in Fig. 5. Following the initial 20-sec association interval with 2.25 nM [^{3}H]prazosin, receptor-specific binding accounted for 8% of total bound radioligand. Over the course of a subsequent 15-min incubation in buffer containing excess phentolamine at 37°, nonspecifically bound [^{3}H]prazosin was preferentially removed from the monolayer so that the specific binding signal was increased to 50% of total binding.

Agonist occupancy of the α_1 receptor measured over short time intervals. The kinetic strategy described above was employed to measure the concentration dependence for agonist occupancy of the α_1 receptor by competition with the initial rate of [^{3}H]prazosin association. Data for epinephrine, norepinephrine, and phenylephrine are shown in Fig. 6. The competition isotherms were fit to a biphasic distribution of receptor affinities for agonist according to Eq. 4.

$$Y = X_H\left(\frac{A}{A + K_{D,H}}\right) + X_L\left(\frac{A}{A + K_{D,L}}\right) \quad (4)$$

Here Y represents the fractional inhibition of the initial rate of [^{3}H]prazosin binding by the agonist concentration, A. X_H and $K_{D,H}$ refer to the fractional population and dissociation constant of the component of higher apparent agonist affinity, while X_L and $K_{D,L}$ denote the corresponding terms for the lower affinity component. The sum of X_H and X_L was constrained to unity. Estimated parameter values are summarized in Table 3. The high affinity component constitutes the major fraction of agonist binding for each agonist. The rank order of the high affinity binding for the three agonists corresponds to the results obtained in the functional studies, namely, E ≥ NE > PE. The dissociation constant of the high affinity component of binding for each agonist correlates well with the effective concentration range for the functional response. A minor component of agonist binding occurs at substantially lower agonist concentration.

The effect of prolonged agonist exposure on receptor affinity was examined by conditioning the receptor with specified agonist concentrations for 30 min prior to measuring agonist competition with [^{3}H]prazosin association. A constant agonist concentration was maintained throughout the conditioning and assay intervals. These data are also presented in Fig. 6. Exposure to agonist for 30 min causes a slight reduction in affinity for the dominant fraction of receptor in the high affinity state.

The effect of prior agonist exposure upon receptor number. The inhibition of prazosin binding by agonist in Fig. 6 could reflect agonist-induced alterations in receptor number as well as the receptor's affinity for agonists. To examine this possibility we performed parallel measurements on α_1 receptor number and the kinetics of prazosin association following either 30 min or 2.5 hr of epinephrine exposure, as shown in Fig. 7. Rates of [^{3}H]prazosin association after removal of the epinephrine are shown in Fig. 7A and were calculated relative to the number of receptors measured in companion equilibrium experiments in Fig. 7B. Exposure of BC3H-1 cells to epinephrine concentrations <10 μM for either 30 min or 2.5 hr followed by rapid removal of the agonist had little effect on the initial rate of [^{3}H]prazosin binding (Fig. 7A). The decline in initial rate of [^{3}H]prazosin binding following exposure to high epinephrine concentrations (>10 μM) may reflect residual agonist which

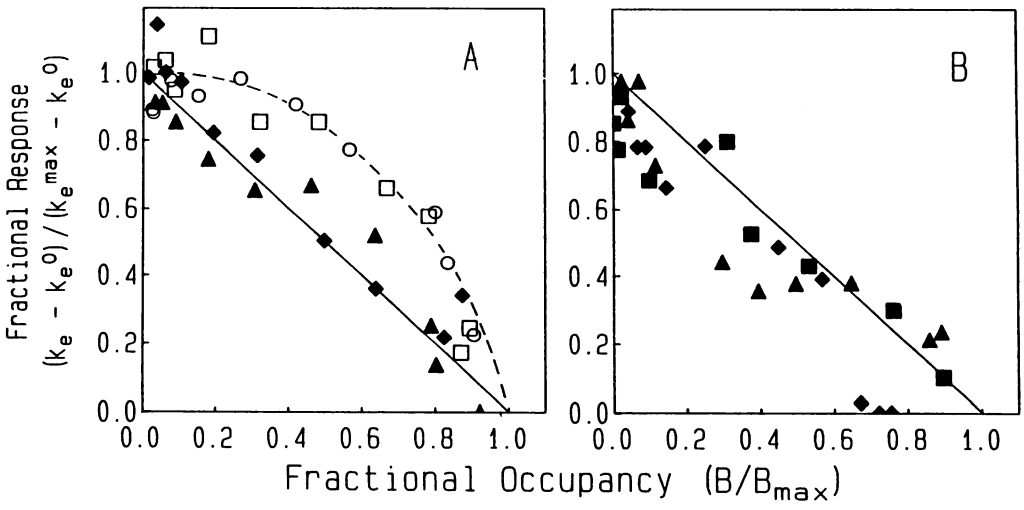

Fig. 4. Occupation and activation of α_1-adrenergic receptors by [^{3}H]prazosin. Replicate cultures were incubated for 2.5 hr with specified [^{3}H]prazosin concentrations. At each prazosin concentration we measured total binding, nonspecific binding (defined by 10 μM phentolamine), and residual $^{45}Ca^{2+}$ efflux response in the presence of the indicated agonist concentrations using parallel cultures previously equilibrated with $^{45}Ca^{2+}$. Scatchard analysis of the binding data yielded the following values for [^{3}H]prazosin: K_D = 50.0 ± 25.7 pM and B_{max} =149 ± 22 fmol/mg of protein (12 determinations). Data from replicate experiments at indicated agonist concentrations are shown. A. Epinephrine: ▲, ◆, 1 μM; ○, □, 6 μM. B. Norepinephrine: ■, ◆, ▲, 1 μM. The *solid line* illustrates the linear behavior expected for a system lacking receptor reserves.

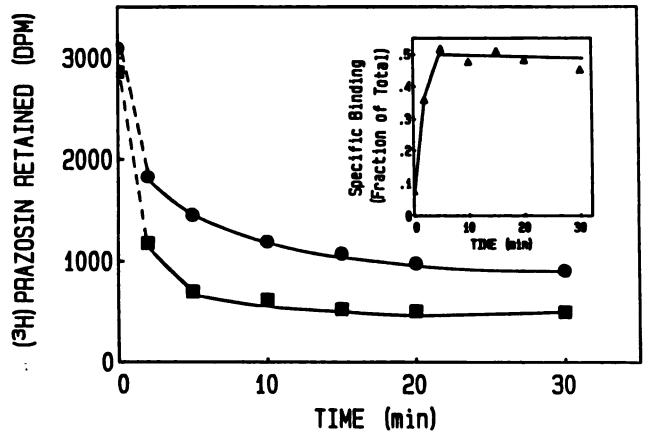

Fig. 5. Kinetics of [^{3}H]prazosin dissociation following an initial interval of association. [^{3}H]Prazosin (2.24 nM) was allowed to associate with BC3H-1 cells for 20 sec in the absence (total binding, ●) or presence (nonspecific binding, ■) of 10 μM phentolamine. This solution was rapidly washed away and replaced with a fresh solution containing 10 μM phentolamine, and the dissociation of [^{3}H]prazosin was measured at the indicated times. *Inset*: Specific [^{3}H]prazosin binding was calculated at each time point in the dissociation curve as the difference between total and nonspecific binding and expressed as a fraction of total [^{3}H]prazosin binding. The data are representative of two experiments.

was not effectively removed during the wash interval prior to prazosin addition. Fig. 7B shows that the total number of [^{3}H] prazosin-binding sites was similarly unchanged following 30 min epinephrine exposure, whereas increasing the exposure interval to 2.5 hr resulted in a decline in receptor number which depended on the conditioning epinephrine concentration. Based on the data in Fig. 7, we conclude that the agonist competition isotherms following 30 min agonist exposure in Fig. 6 reflect slightly reduced affinity of the receptor for agonist during this conditioning interval. Fig. 7B also suggests that a more prolonged exposure of 2.5 hr might have more dramatic effects on receptor properties.

The influence of prolonged agonist exposure on agonist occupation of the receptor. To examine further the effects of agonist exposure on receptor affinity, BC3H-1 cells were conditioned with specified epinephrine concentrations for 2.5 hr, and the initial rate of [^{3}H]prazosin association was then measured while maintaining the same epinephrine concentration. Receptor number following agonist exposure was measured in parallel cultures as described in Fig. 7 and the calculated rates reflect agonist-induced alterations in receptor number. Results are shown in Fig. 8. When these data were fit to Eq. 4, the estimated values for $K_{D,H}$ and $K_{D,L}$ were $3.53 \pm 0.09 \times 10^{-7}$ M and $7.20 \pm 0.04 \times 10^{-5}$ M, respectively, with corresponding amplitudes of 0.46 ± 0.05 and 0.54 (Table 3). Thus, the receptor population redistributes toward reduced apparent agonist affinity during this prolonged period of agonist exposure. A bimodal distribution of affinities is still observed, and the estimated values for $K_{D,H}$ and $K_{D,L}$ correspond reasonably well with values measured following shorter durations of agonist exposure. However, the majority of the receptor population now displays low agonist affinity with a smaller fraction of receptors still competent to bind agonist with high affinity. Thus, the smaller alterations in receptor affinity for agonist seen after 30 min are greatly accentuated with a 2.5-hr conditioning interval. This result indicates that the transition in agonist affinity which occurs during conditioning is a comparatively slow process for α_1 receptors. Moreover, Fig. 7 shows that extended agonist exposure alters the number of [^{3}H]prazosin-binding sites as well.

Discussion

The relationship between receptor occupancy and response. The development of classic receptor theory has provided quantitative methods to describe the functional response in terms of agonist occupancy and receptor number. We have used two experimental protocols to examine this relationship in BC3H-1 cells. First, the concentration dependence for agonist activation was compared in the presence and absence of pseudo-irreversible antagonism by prazosin to calculate the agonist dissociation constant and the fraction of activatable receptors subserving specified responses. Second, prazosin occupancy of the receptor was measured directly by radioligand binding techniques and correlated with the residual response to a test agonist concentration. We previously validated the use of prazosin for temporary receptor inactivation by comparison

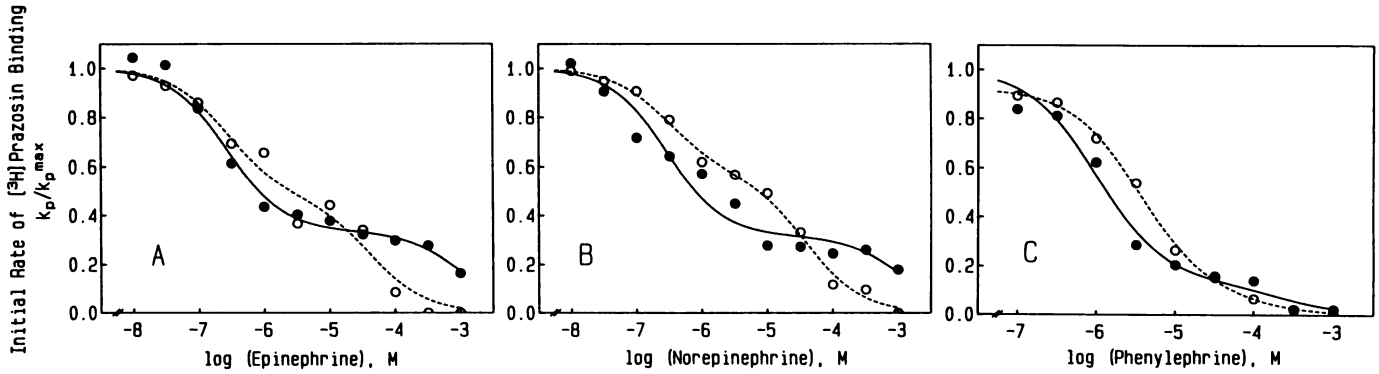

Fig. 6. Concentration dependence for agonist competition with the initial rate of [^{3}H]prazosin association. Agonist affinities were measured by competition with initial rates of [^{3}H]prazosin association over a 20-sec interval as outlined in Experimental Procedures. ●, cultures incubated for 30 min in buffer containing no agonist addition followed by initial rate measurements in the presence of the specified agonist concentrations plus [^{3}H] prazosin. ○, cultures which received an initial 30-min incubation in buffer containing the specified agonist concentrations followed by initial rate measurements in the presence of [^{3}H]prazosin plus the specified agonist concentration. Nonspecific binding was determined from cultures which received 10 μM phentolamine during the 20-sec association interval. Rate constants were calculated by pseudo-first order kinetics and expressed relative to the control rate of [^{3}H]prazosin association in the absence of agonist. A, epinephrine (n = 4–7); B, norepinephrine (n = 4); C, phenylephrine (n = 2).

TABLE 3

Parameters for agonist occupation of α_1-adrenergic receptors in BC3H-1 cells

Data from indicated figures were fit by nonlinear regression to two binding affinities as described in Eq. 4. $K_{D,H}$ and $K_{D,L}$ refer to high and low affinity agonist binding components and X_H and X_L indicate their respective amplitudes.

A. Agonist competition with initial rate of [^{3}H]prazosin association with simultaneous addition of agonist and [^{3}H]prazosin (Fig. 6):

Agonist	$K_{D,H}$	X_H	$K_{D,L}$	X_L
	M		*M*	
Epinephrine	$2.81 \pm 0.05 \times 10^{-7}$	0.67 ± 0.03	$1.09 \pm 0.10 \times 10^{-3}$	0.33
Norepinephrine	$2.93 \pm 0.06 \times 10^{-7}$	0.69 ± 0.04	$1.15 \pm 0.15 \times 10^{-3}$	0.31
Phenylephrine	$1.02 \pm 0.02 \times 10^{-6}$	0.87 ± 0.06	$2.23 \pm 0.42 \times 10^{-4}$	0.13

B. Agonist competition with initial rate of [^{3}H]prazosin association following 2.5 hr agonist conditioning (Fig. 8):

Epinephrine	$3.53 \pm 0.09 \times 10^{-7}$	0.46 ± 0.05	$7.20 \pm 0.04 \times 10^{-5}$	0.54

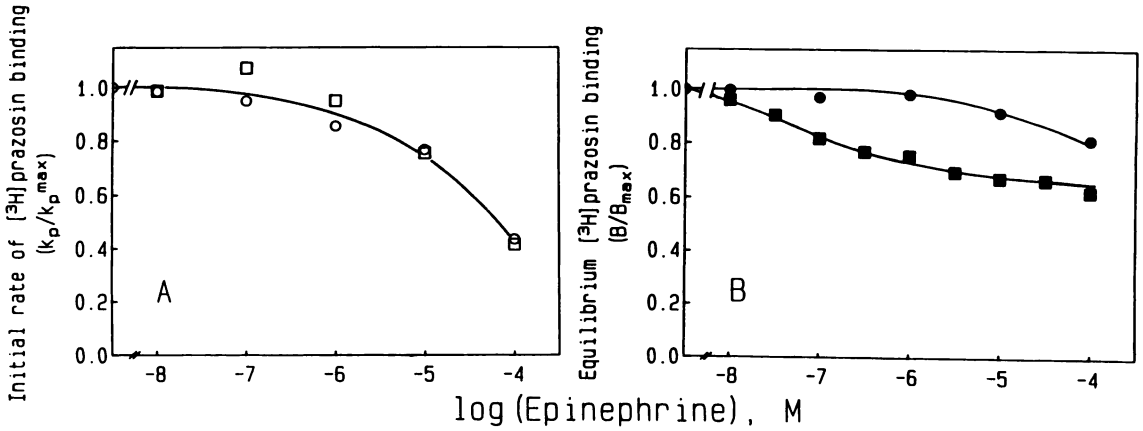

Fig. 7. Initial rate and equilibrium [^{3}H]prazosin binding following intervals of epinephrine exposure. Cultures were incubated with physiological buffer containing the specified agonist concentration for either 30 min (●, ○) or 2.5 hr (■, □). Following this interval, the conditioning solution was removed, the culture was rapidly washed to remove the agonist, and a fresh solution containing [^{3}H]prazosin was added to measure either initial rate (A, 3 nM [^{3}H]prazosin for 20 sec) or equilibrium (B, 500 pM [^{3}H]prazosin for 20 min) radioligand binding. Experimental values are expressed relative to control cultures which had been incubated with physiological buffer containing no agonist additions during the conditioning interval. Nonspecific binding was defined using 10 μM phentolamine (n = 3).

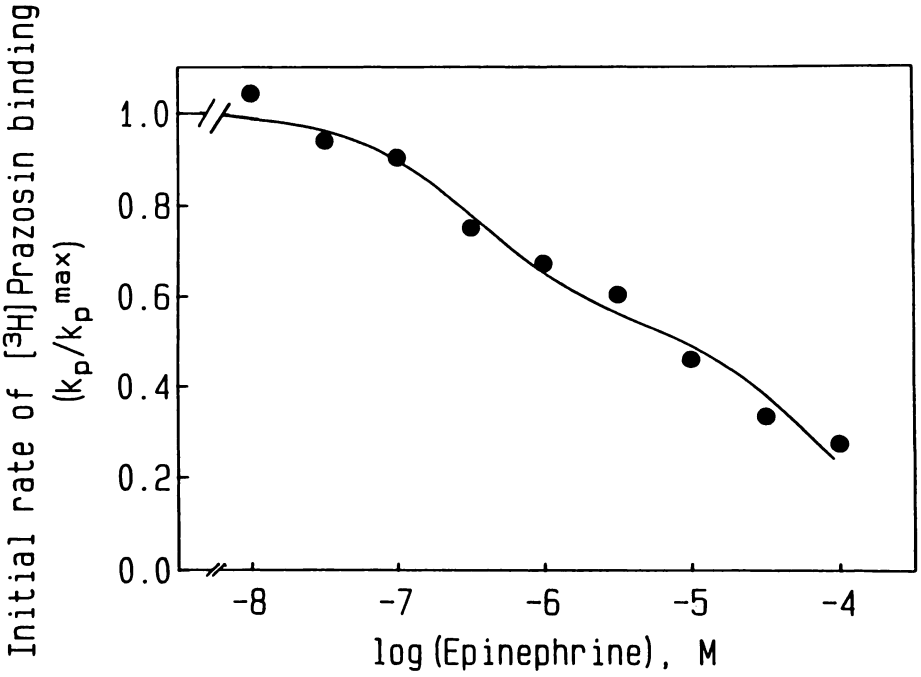

Fig. 8. Epinephrine competition with the initial rate of [^{3}H]prazosin association following a 2.5-hr conditioning interval. Cultures were incubated with buffer containing specified epinephrine concentrations for 2.5 hr. Following this interval, the initial rate of [^{3}H]prazosin association was measured in the presence of the same epinephrine concentration used for conditioning. Equilibrium [^{3}H]prazosin binding following epinephrine exposure was measured in sister cultures as described in Fig. 7. First order rate constants of prazosin association at each epinephrine concentration were calculated relative to the total number of [^{3}H]prazosin-binding sites determined from the corresponding equilibrium measurement (n = 3).

with the alkylating agent phenoxybenzamine (9). As shown in Figs. 3 and 4 and Table 2, the two methods give self-consistent results. Evidence was adduced for a modest receptor reserve resulting in only 2- to 3-fold differences between EC_{50} and K_A values for the two agonists. Our previous studies using phenylephrine revealed a unitary relationship between receptor occupancy and response indicating the lack of appreciable receptor reserves for this agent, which would be classed as a partial agonist in this system. Fig. 1 shows that the maximal response elicited by phenylephrine is slightly less than that for epinephrine or norepinephrine, consistent with the lower efficacy observed in the receptor inactivation experiments. Thus, coupling between adrenergic agonists and response in the BC3H-1 system behaves in accordance with the predictions of receptor theory.

Previous studies measuring the relationship between α_1 receptor number and contractile response in intact smooth muscle have indicated the presence of more substantial receptor reserves (15–19). Moreover, when receptors have been quantitated directly by radioligand binding techniques in tissue homogenates, the number of available receptors has been significantly greater than the prediction from the inactivation experiments. For example, Minneman and Abel (16) found that a phenoxybenzamine treatment which predicted 93% α_1 receptor inactivation based on inhibition of vas deferens contractile responses in fact inactivated only 60% of the available radioligand-binding sites in the tissue homogenate. Similar results were obtained from measurements of α_1 receptor-elicited $^{45}Ca^{2+}$ efflux in primary cultures of vascular smooth muscle cells relative to receptor numbers measured in cellular homogenates (20, 21). We previously suggested that the discrepancy between BC3H-1 cells and intact tissue might relate to the structural and functional complexity inherent to intact smooth muscle or to differential amplification intervening between receptor occupancy and $^{45}Ca^{2+}$ efflux versus muscle contraction (9). A further possibility would be that measurements of α_1 receptor numbers in cellular homogenates overestimate the functionally coupled receptors which are actually contributing to the observed response. This possibility will be addressed in more detail below. We would emphasize that the receptor inactivation experiments reported here measure receptor number and response under identical conditions in genetically homogeneous monolayer cultures of intact cells, assuring uniform exposure of receptors to agonists and antagonists. Under these conditions the BC3H-1 system behaves in accordance with the predictions of receptor theory, and receptor reserves are minimal. It will be of interest to measure the relationship between receptor occupancy and more distal Ca^{2+}-dependent responses in BC3H-1 cells in order to determine whether enhanced receptor reserves are observed with respect to the functional endpoints in the response cascade.

The concentration dependences for agonist occupation and functional response. The functional studies described above quantitate parameters of receptor activation upon acute agonist challenge and allow for secondary calculation of agonist occupancy. To complement the receptor inactivation experiments, we have developed procedures to measure agonist occupancy experimentally within the short time intervals of the functional assays and we have evaluated the influence of prolonged agonist exposure upon the receptor's ligand recognition capabilities. We find that in the absence of prior agonist exposure, the major fraction of receptors (70–90%) exhibits agonist affinities which correlate closely with the dissociation constants calculated from the functional studies. This correspondence further confirms the absence of substantial receptor reserves in this system. An additional minor fraction of receptors (10–30%) is present which exhibits substantially lower agonist affinity and would not be expected to be activated at the agonist concentrations employed in the functional experiments.

Cellular Ca^{2+} homeostasis and α_1 receptor activation. α_1-Adrenergic receptors act within the framework of cellular Ca^{2+} homeostasis to shift the disposition of intracellular Ca^{2+}. In the BC3H-1 system, the initial sequelae of receptor activation are a dominant mobilization and efflux of intracellular Ca^{2+}. A smaller enhancement of Ca^{2+} entry also accompanies receptor activation. Separate unidirectional $^{45}Ca^{2+}$ flux measurements provide an estimate of the contributions of these two pathways. The initial rates of the fluxes and their respective amplitudes are most accurately determined by fitting the integrated time course to multiexponential kinetics. When this analysis is performed, we find that agonist-elicited efflux exceeds influx by a factor of 3–4 (Fig. 2; see also Refs. 7 and 10). This result is in quantitative accord with the experimental finding that α_1-adrenergic agonists elicit a net 30–40% decrease in cellular Ca^{2+} content (7). However, the total fraction of cellular Ca^{2+} mobilized by agonist relative to intracellular volume exceeds the concentration of free intracellular Ca^{2+} measured directly with quin 2 (8). This discrepancy likely reflects substantial cycling of intracellular Ca^{2+} between bound and free states during the activation process. Such buffering would blunt the increase in free Ca^{2+} despite enhanced exchange from intracellular stores. A further possibility would be that elevations in free Ca^{2+} may occur in localized regions within the cell rather than being distributed uniformly throughout the cytoplasm.

The influence of prior agonist exposure on receptor properties. When BC3H-1 cells are exposed to agonist over prolonged intervals, the receptor population slowly redistributes toward a state exhibiting altered ligand-binding properties. This progression is evident over a 30-min interval of exposure to physiologically effective agonist concentrations but certainly has not proceeded to completion. These results are consistent with our previous study which showed that the α_1 receptor remains functionally responsive over this interval of agonist exposure (10). Similar findings have been reported for hepatocytes (22) and canine aorta (17), where α_1 receptor-mediated responses are maintained during prolonged agonist application.

Extending the period of agonist exposure up to 2.5 hr reduces the number of receptors detected by prazosin binding as well as their ability to associate with agonists, so that those receptors which remain exhibit reduced affinity for agonist. A significant proportion of receptors with low agonist affinity is detectable within 20 sec of agonist application, whereas the overall rate of receptor conversion during agonist exposure is slow, suggesting that this form of the receptor may preexist the addition of agonist. The original fraction of high affinity receptors is preferentially lost during prolonged agonist incubation by conversion to a state with reduced agonist affinity and by an outright reduction in number. The reduction in receptor number is not easily reversed; it persists during the 20-min interval used to measure receptor number in Fig. 8. At present

the fate of the receptors lost during agonist occupancy is not known. These data cannot distinguish whether the disappearance of receptors proceeds through the low affinity form as an obligatory intermediate or whether receptor loss from the high affinity fraction occurs by an independent process.

These results on intact cells are consistent with previous studies on agonist-induced down-regulation of α_1 receptors on BC3H-1 cells (23) and on cultured vascular smooth muscle cells (21, 24), where long-term agonist exposure progressively reduced the number of α_1 receptors measured in cellular homogenates. A report by Sladeczek *et al.* (25) suggested that high affinity agonist binding could be detected in equilibrium binding experiments with intact BC3H-1 cells at 4° or with BC3H-1 cell membranes at 4° and 37°, whereas intact cells at 37° revealed substantially reduced apparent agonist affinities. Although quantitative comparisons between our studies which measure receptors in intact cells and the previous studies are difficult, those worker's observations would suggest that the loss of receptors induced by agonist is inhibited in broken cell preparations and at reduced temperatures, as would be expected for a process requiring cellular energy or structural integrity.

Recently, Schwarz *et al.* (26) have employed initial rate methods to measure agonist affinity for α_1 receptors in isolated hepatocytes. In agreement with our data, the hepatic receptor exhibits a major fraction (70%) of high agonist affinity which correlates well with physiologically effective agonist concentrations and a minor component (30%) of substantially lower agonist affinity. However, agonist exposure in this system rapidly ($t_{1/2} = 1$ min) converts the receptor population to reduced affinity for agonist. We find no evidence for such a rapid conversion in BC3H-1 cells. Moreover, it is difficult to reconcile transiently occurring states of receptor affinity with the sustained physiological responses elicited by α_1 receptors in liver (22).

The heterogeneities and agonist-induced alterations in receptor properties reported here have implications for attempts to quantitate receptor occupancy-response relationships. Clearly, estimates of agonist occupancy derived from equilibrium competition experiments may suffer from multiple processes occurring during the assay interval. The kinetic measurements which we describe here avoid these uncertainties by measuring occupancy and response under identical conditions over short time intervals and, moreover, provide quantitative insight into the influence of agonist exposure on receptor state.

We can consider BC3H-1 cells as a simplest case where, beginning with a naive population of receptors, we have issued an agonist challenge and measured the activation of cellular responses and subsequent alteration in the distribution and metabolism of receptors. Smooth muscle *in vivo* represents a far more complex situation where the cell is constitutively exposed to neurotransmitter. In this situation a substantially larger proportion of the receptor population might exist in a cryptic or unresponsive form than occurs with BC3H-1 cells. This possibility would account for the discrepancy between receptor numbers predictable from receptor theory versus direct measurements in radioligand binding experiments. Moreover a common observation with alpha$_1$ receptors has been that a major proportion of the maximum response occurs over a small range of receptor occupancy, whereas near complete occupancy is required to evoke the maximum response. Thus, both Sastre *et al.* (17) and Besse and Furchgott (18) found that ≤10% receptor occupancy evoked a half-maximal contractile response, yet virtually full occupancy was required to elicit the maximum response.

In summary, the α_1 receptor in BC3H-1 cells emerges as a multifunctional entity capable of eliciting both Ca^{2+} mobilization and entry. Parameters for agonist occupation correlate well with the functional response, and the system may be employed as a simple example of a Ca^{2+}-regulatory receptor capable of supporting a sustained response. Prolonged agonist exposure results in an altered distribution of α_1 receptors, and it will be of interest to examine the consequences of this process on the regulation of cellular Ca^{2+}. Finally, the methods and approaches developed here for quantitating receptor reserves should be directly applicable to studies on primary cultures or freshly dissociated smooth muscle cells.

Acknowledgments

The authors are grateful to Richard Hughes and Kelly Ambler for helpful discussions during the course of this work, to Harvey Motulsky for assistance with data analysis and curve fitting, and to Kathy Knight and Miriam Manning for assistance in preparing the manuscript.

References

1. Bolton, T. B. Mechanisms of action of transmitters and other substances on smooth muscle. *Physiol. Rev.* **59**:606–718 (1979).
2. Furchgott, R. F. The use of beta-haloalkylamines in the differentiation of receptors and in the determination of dissociation constants of receptor-agonist complexes. *Adv. Drug Res.* **3**:21–55 (1966).
3. Weber, M. T., T. David-Pfeuty, and J.-P. Changeux. Regulation of binding properties of the nicotinic receptor proteins by cholinergic ligands in membrane fragments from *Torpedo marmorata*. *Proc. Natl. Acad. Sci. USA* **72**:3443–3447 (1975).
4. Insel, P. A., L. C. Mahan, H. J. Motulsky, L. M. Stoolman, and A. M. Koachman. Time-dependent decreases in binding affinity of agonists for beta adrenergic receptors of intact S-49 cells. *J. Biol. Chem.* **258**:13597–13605 (1983).
5. Weiland, G., B. Georgia, V. T. Wee, C. F. Chignell, and P. Taylor. Ligand interactions with cholinergic receptor-enriched membranes from *Torpedo:* influence of agonist exposure on receptor properties. *Mol. Pharmacol.* **12**:1091–1105 (1976).
6. Rasmussen, H., and P. Q. Barrett. Calcium messenger system: an integrated view. *Physiol. Rev.* **64**:938–984 (1984).
7. Brown, R. D., K. D. Berger, and P. Taylor. Alpha$_1$-adrenergic receptor activation mobilizes cellular Ca^{2+} in a muscle cell line. *J. Biol. Chem.* **259**:7554–7562 (1984).
8. Ambler, S. K. and P. Taylor. Mobilization of intracellular calcium by alpha$_1$-adrenergic receptor activation in muscle cell monolayers. *J. Biol. Chem.* **261**:5866–5871 (1986).
9. Amitai, G., R. D. Brown, and P. Taylor. The relationship between alpha$_1$-adrenergic receptor activation and the mobilization of intracellular Ca^{2+}. *J. Biol. Chem.* **259**:12519–12527 (1984).
10. Brown, R. D., P. Prendiville, and C. Cain. α_1-Adrenergic and H1-histamine receptor control of intracellular Ca^{2+} in a muscle cell line: the influence of prior agonist exposure on receptor responsiveness. *Mol. Pharmacol.* **29**:531–539 (1986).
11. Brown, R. D., K. D. Berger, D. Button, and P. Taylor. The relationship between alpha$_1$ adrenergic receptor occupancy and regulation of intracellular Ca^{2+} in BC3H-1 muscle cells. *Fed. Proc.* **45**:1690, abstr. 1225 (1986).
12. Mahan, L. C., and P. A. Insel. Use of superoxide dismutase and catalase to prevent catecholamines from oxidation in tissue culture studies. *Anal. Biochem.* **136**:208–216 (1984).
13. Ambler, S. K., R. D. Brown, and P. Taylor. The relationship between phosphatidylinositol metabolism and mobilization of intracellular calcium elicited by alpha$_1$-adrenergic receptor stimulation in BC3H-1 muscle cells. *Mol. Pharmacol.* **26**:405–413 (1984).
14. Brading, A. F. *Ionic distribution and mechanisms of transmembrane movements*, in Smooth Muscle (E. Bulbring, A. F. Brading, A. W. Jones, and T. Tomita, eds.). University of Texas Press, Austin, TX, 65–92 (1981).
15. Ruffolo, R. R., Jr. Spare alpha adrenoceptors in the peripheral circulation: excitation coupling. *Fed. Proc.* **45**:2341–2346 (1986).
16. Minneman, K. P., and P. W. Abel. "Spare" alpha$_1$-adrenergic receptors and the potency of agonists in rat vas deferens. *Mol. Pharmacol.* **25**:56–63 (1984).
17. Sastre, A., K. K. Griendling, M. M. Rusher, and W. R. Milnor. Relation between alpha$_1$-adrenergic receptor occupation and contractile response: radioligand binding and physiologic studies in canine aorta. *J. Pharmacol. Exp. Ther.* **229**:887–896 (1984).
18. Besse, J. C., and R. F. Furchgott. Dissociation constants and relative efficacies of agonists acting on alpha adrenergic receptors in rabbit aorta. *J. Pharmacol. Exp. Ther.* **197**:66–78 (1976).

19. Sheys, E. M., and R. D. Green. A quantitative study of alpha-adrenergic receptors in the spleen and aorta of the rabbit. *J. Pharmacol. Exp. Ther.* **197**:66–78 (1976).
20. Colucci, W. S., T. A. Brock, M. A. Gimbrone, Jr., and R. W. Alexander. Nonlinear relationship between alpha$_1$-adrenergic receptor occupancy and norepinephrine-stimulated calcium flux in cultured vascular smooth muscle cells. *Mol. Pharmacol.* **27**:517–524 (1985).
21. Colucci, W. S., and R. W. Alexander. Norepinephrine-induced alteration in the coupling of alpha$_1$-adrenergic receptor occupancy to calcium efflux in aortic smooth muscle cells. *Proc. Natl. Acad. Sci. USA* **83**:1743–1746 (1986).
22. Morgan, N. G., E. A. Shuman, J. H. Exton, and P. F. Blackmore. Stimulation of hepatic glycogenolysis by alpha$_1$- and alpha$_2$-adrenergic agonists. *J. Biol. Chem.* **257**:13907–13910 (1982).
23. Hughes, R. J., and P. A. Insel. Agonist-mediated regulation of α_1 and β_2-adrenergic receptor metabolism in a muscle cell line, BC3H-1. *Mol. Pharmacol.* **29**:521–530 (1986).
24. Wikberg, J. E. S., M. Akers, M. G. Caron, and P.-O. Hagen. Norepinephrine-induced down regulation of alpha$_1$-adrenergic receptors in cultured rabbit aorta smooth muscle cells. *Life Sci.* **33**:1409–1417 (1983).
25. Sladeczek, F., J. Bockaert, and J.-P. Mauger. Differences between agonists and antagonist binding to alpha$_1$-adrenergic receptors of intact and broken cell preparations. *Mol. Pharmacol.* **24**:392–397 (1983).
26. Schwarz, K. R., S. M. Lanier, E. A. Carter, R. M. Graham, and C. J. Homcy. Transient high affinity binding of agonists to alpha$_1$-adrenergic receptors in intact liver cells. *FEBS Lett* **187**:205–210 (1985).

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