GREATER TENSION IS DEVELOPED AT THE SAME LEVEL OF CYTOSOLIC Ca^{2+} CONCENTRATION IN THE PRESENCE OF CLONIDINE, AN ADRENERGIC PARTIAL AGONIST, THAN IN THE PRESENCE OF NOREPINEPHRINE

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Fura 2-loaded thoracic aorta strips from rabbits were used. Norepinephrine and clonidine induced an increase in cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) and in muscle tension in a concentration-dependent manner. A positive correlation between $[\text{Ca}^{2+}]_i$ and tension development due to norepinephrine and clonidine was noted in muscle strips both untreated and treated with phenoxybenzamine (PBZ). As the total receptor population was reduced by the PBZ treatment, norepinephrine acted as a partial agonist in the treated strips. The slope of a regression line for norepinephrine in the untreated muscle strips was significantly smaller than the slopes for the same drug in PBZ-treated strips and for clonidine, suggesting that norepinephrine in the PBZ-treated strips and clonidine induced a greater tension at same $[\text{Ca}^{2+}]_i$ than did norepinephrine in the untreated strips.

KEYWORDS — cytosolic Ca^{2+} concentration, $[\text{Ca}^{2+}]_i$; muscle tension; adrenergic partial agonist; clonidine; norepinephrine; rabbit aorta

INTRODUCTION

It is well known that there are spare receptors for a full agonist, an agonist with high efficacy or intrinsic activity of 1, and they give a maximal response when only a small proportion of the total receptor population is occupied, while a partial agonist, an agonist with low efficacy or intermediate intrinsic activity, produces a small response, even when the total receptor population is occupied. A reliable method to

measure smooth muscle contraction simultaneously with fluorescent dye Ca^{2+} signals in vascular smooth muscle using dual wavelength excitation has been introduced by Ozaki et al.¹⁾ Recently, Sato et al.²⁾ reported the existence of a positive correlation between cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) and tension development in the presence of K^+ or norepinephrine, and showed that greater tension development was induced at the same level of $[\text{Ca}^{2+}]_i$ in the presence of norepinephrine than in the presence of K^+ . In this paper we tested the relationship between changes in tension development and in $[\text{Ca}^{2+}]_i$ in the presence of norepinephrine, a full agonist, and clonidine, a partial agonist.

MATERIALS AND METHODS

The thoracic aorta was isolated from male Albino rabbits (2-3 kg) and cut into spinal strips, 2-3 mm wide and 7-10 mm long. The endothelium was removed by gently rubbing the intimal surface with a filter paper moistened with a physiological salt saline (PSS) which contained NaCl 118.1, KCl 4.5, CaCl_2 2.5, MgCl_2 1.2, NaHCO_3 25.0 and glucose 11.0 mM. In experiments measuring $[\text{Ca}^{2+}]_i$, the PSS was supplemented with 10 μM ethylenediamine-N,N,N',N'-tetraacetic acid disodium salt (EDTA Na_2) to mask the possible effects of heavy metal ions.³⁾ The PSS contained propranolol (10^{-6} M), yohimbine (3×10^{-7} M), desmethylinipramine (10^{-7} M) and normethanephine (10^{-6} M) to block alpha-2-and beta-adrenoceptors and to inhibit neural and nonneural uptakes of catecholamine, respectively. The muscle strips were placed horizontally in an organ bath, which was filled with PSS kept at 37°C and gassed with a mixture of 95% O_2 5% CO_2 . The mechanical responses to drugs were recorded isometrically under a resting tension of 0.5 g. $[\text{Ca}^{2+}]_i$ was measured with a fluorescent Ca^{2+} indicator, fura 2. For loading with fura 2, the muscle strips were incubated with 5 μM fura 2 pentaacetoxymethylester (fura 2/AM) in the PSS for 6 hr at room temperature in the dark. A noncytotoxic detergent, Cremophor EL (0.2%), was added to increase the solubility of fura 2/AM. After the fura 2-loading, the muscle strips were rinsed with the PSS for 15 min; experiments were performed within 1 hr thereafter. Fura 2- Ca^{2+} signal and muscle tension were measured simultaneously with a fluorimeter (CAF-100, Japan Spectroscopic) according to the method of Ozaki et al.¹⁾ The ratio of the emitted fluorescent light with a 500 nm filter due to

excitation at 340 nm against that at 380 nm was calculated automatically from successive illumination periods (48 Hz) and referred to as R340/380; this was used to monitor the relative $[\text{Ca}^{2+}]_i$. The total receptor population is known to be less in phenoxybenzamine (PBZ)-treated muscles than in normal untreated ones, and a full agonist, norepinephrine is known to act as a partial agonist.⁴⁻⁷⁾ Therefore, in some experiments the muscle strips were treated with PBZ (10^{-7} M) for 7.5 min. They were then allowed to equilibrate for 15 min, with repeated washing every 5 min and used as test tissues.

Fig. 1. A: Effects of norepinephrine and clonidine on R340/380 and tension. (a) norepinephrine (NE; 10^{-5} M) in the untreated strip, (b) clonidine (Clo; 3×10^{-6} M) in the untreated strip and (c) norepinephrine (NE; 10^{-5} M) in the strip treated with PBZ (10^{-7} M). Upper and lower traces: R340/380 and tension, respectively. K: 70mM K^+ . W: washing with the PSS. B: Concentration response relationships of norepinephrine and clonidine in R340/380 and tension. Abscissa: logarithm of molar concentration of drug, and ordinate: 100% represents 70 mM K^+ -induced increases in tension and R340/380. ●, ○: norepinephrine in the untreated strips, ▲, △: norepinephrine in the PBZ-treated strips and ■, □: clonidine in the untreated strips. Closed symbols: tension and open symbols: R340/380. Each value is presented as a mean with S.E. of 4 experiments.

RESULTS

Norepinephrine (10^{-5} M) caused a rapid increase in $[Ca^{2+}]_i$ (R340/380), which declined gradually to a level. The vascular smooth muscle tension also increased in response to norepinephrine (Fig. 1A (a)). Clonidine (3×10^{-6} M) (Fig. 1A (b)) and norepinephrine (lower than 10^{-6} M) increased $[Ca^{2+}]_i$ (R340/380) gradually, and this was accompanied by a low increase in muscle tension. Similar results were obtained in the smooth muscle strips treated with PBZ (10^{-7} M) (Fig. 1A (c)). To obtain reference values, R340/380 and tension in 70 mM K^+ -PSS were measured by replacing NaCl with equimolar KCl to give a final concentration of 70 mM K^+ . Seventy mM K^+ caused a rapid increase in $[Ca^{2+}]_i$ (R340/380), followed by a slow and slight decrease. Vascular smooth muscle strip caused a tonic contraction in 70 mM K^+ -PSS (Fig. 1A).

Both $[Ca^{2+}]_i$ (R340/380) and muscle tension were increased by norepinephrine and clonidine in a concentration-dependent manner. Similar results with norepinephrine were obtained in the strips treated with PBZ

Fig. 2. Relationship between R340/380 and tension development in the presence of various concentrations of norepinephrine and clonidine. ●: norepinephrine in the untreated strips, ▲: norepinephrine in the PBZ-treated strips and ■: clonidine in the untreated strips. Correlation coefficient is 0.988 ($P < 0.05$) and 0.975 ($P < 0.05$) for norepinephrine in the untreated and PBZ-treated strips, and 0.990 ($P < 0.05$) for clonidine in the untreated strips. Each value is presented as a mean with S.E. of 4 experiments.

(Fig. 1B). Figure 2 shows the existence of a positive correlation between $[Ca^{2+}]_i$ (R340/380) and tension development due to clonidine and norepinephrine in strips both untreated and treated with PBZ. A regression line was obtained from all the points for each drug and from zero point for R340/380. Slopes of regression are 1.16 ± 0.15 and 3.20 ± 0.51 for norepinephrine in the untreated and PBZ-treated strips and 3.16 ± 0.33 for clonidine (each value is a mean with S.E. of 4 observations). The slope for norepinephrine (1.16) in the untreated strips was significantly smaller ($P < 0.05$) than the other two values. Furthermore, the pA_2 value (10.6 ± 0.13), significantly differ from that (9.85 ± 0.16) against clonidine suggesting that the sites of action of both the agonist are α_1 -adrenoceptors, as reported in rat aortic strips.⁸⁾

DISCUSSION

The receptor occupancy-response curves for full agonists, agonists with higher intrinsic activity, were rectangular hyperbolic, while the curves for partial agonists with moderately higher intrinsic activity were flattened. The partial agonists with lower intrinsic activity produced linear occupancy-response relationships.⁵⁻⁷⁾ The amount of active receptors was smaller in the PBZ-treated muscle preparations than in the untreated ones, and the efficacy of the full agonist was less in the former than in the latter.⁵⁻⁷⁾ In the treated muscle preparations the full agonist also produced a linear occupancy-response relationship.⁵⁻⁷⁾ These results suggest that the receptor occupancy-response relationship changes according to the intrinsic activity or efficacy of the agonist. The slope of the regression line for norepinephrine was significantly lower than the slope for clonidine in this study. Thus, greater tension may be induced at the same level of $[Ca^{2+}]_i$ (R340/380) in the presence of clonidine than in the presence of norepinephrine. Furthermore, the slope for norepinephrine was significantly steeper in the PBZ-treated than in the untreated muscle strips, suggesting that greater tension is induced in the treated strips. In conclusion, norepinephrine in the PBZ-treated strips and clonidine, a partial agonist, induced a greater tension at the same $[Ca^{2+}]_i$ than norepinephrine in the untreated strips, though norepinephrine and clonidine activated the same α_1 -adrenoceptors in this study. Activation mechanisms of contractile system by norepinephrine is

considered to be different from those by clonidine, as suggested from the facts that the receptor occupancy relationships for the full agonists are different from those for the partial agonists.⁵⁻⁷⁾ There is still much research remaining to be done in this field.

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