Dissociation Constant of the Norepinephrine-Receptor Complex in Normotensive and Hypertensive Rats

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ABSTRACT

Previous reports have suggested that smooth muscle obtained from the thoracic aorta of spontaneously hypertensive rats is less responsive to vasoconstrictive agents than that obtained from normotensive rats. The present study was undertaken to determine whether the responsiveness of aortic muscles from normotensive and spontaneously hypertensive rats correlates with a difference in the affinity of the adrenergic receptors for norepinephrine and whether antihypertensive therapy alters the affinity of the adrenergic receptors for norepinephrine. The affinity of the adrenergic receptors for norepinephrine was determined by computing the dissociation constant of the norepinephrine-receptor complex ($K_{nR}$). The values computed for $K_{nR}$ in aortic muscles from normotensive and spontaneously hypertensive rats that had received no antihypertensive therapy were $1.07 \times 10^{-7}$M and $1.17 \times 10^{-7}$M, respectively. The values computed for $K_{nR}$ in aortic muscles from normotensive and spontaneously hypertensive rats that had received antihypertensive therapy were $1.38 \times 10^{-7}$M and $1.29 \times 10^{-7}$M, respectively. The differences in these values for $K_{nR}$ are not significant. These results indicate that the difference in the contractility of aortic muscles from normotensive and spontaneously hypertensive rats is not related to an alteration in the affinity of the adrenergic receptors for norepinephrine and that the affinity of the adrenergic receptors for norepinephrine is not altered by antihypertensive therapy. Thus, it appears that the etiology of hypertension cannot be directly correlated with a difference in the affinity of the adrenergic receptors for norepinephrine.

Although essential hypertension has been the subject of numerous studies, the etiology of this disease is not completely understood. It has been postulated that essential hypertension develops secondary to an increase in resistance at the arteriolar level (1–3). This increase in resistance may be due to one or a combination of the following three mechanisms. First, there may be an enhanced vasoexcitatory influence (neurogenic, blood-borne, or local) to which the arterioles respond normally (4–7). Second, a change in the inherent responsiveness of the vascular smooth muscle itself may cause an increase in resistance in response to normal vasoexcitatory influences (8–11). Finally, a structural change in the vascular smooth muscle may result in an increase in resistance at normal levels of smooth muscle activity (12–14).

An abnormality in the interaction between receptors located in the membrane of vascular smooth muscle and the adrenergic neurotransmitter norepinephrine could contribute to the etiology and the maintenance of essential hypertension. This possibility was investigated in the present paper by computing the dissociation constant of the norepinephrine-receptor complex ($K_{nR}$) in normotensive and spontaneously hypertensive rats. A significant difference in $K_{nR}$ would indicate an alteration in the affinity of the adrenergic receptors for norepinephrine. We also computed $K_{nR}$ in normotensive and spontaneously hypertensive rats that had been maintained on antihypertensive therapy which effectively controlled high blood pressure to determine whether such treatment altered the affinity of the adrenergic receptors for norepinephrine. We also computed $K_{nR}$ in normotensive and spontaneously hypertensive rats that had been maintained on antihypertensive therapy which effectively controlled high blood pressure to determine whether such treatment altered the affinity of the adrenergic receptors for norepinephrine. $K_{nR}$ was calculated for isolated strips of smooth muscle from the aorta of normotensive and spontaneously hypertensive rats. The Wistar strain of spontaneously hypertensive rats developed through selective breeding techniques by Okamoto and Aoki (15) was chosen for this study, because it appears to be a good model for the study of mechanisms involved in essential hypertension. Wistar normotensive rats were used, because they constitute the strain of rats that is generally most homogeneous with respect to the spontaneously hypertensive rats that were employed.

Methods

Male Wistar rats obtained from Carworth Farms were used in this study. The normotensive and spontaneously
hypertensive rats were further subdivided into four groups: (1) spontaneously hypertensive rats that received no antihypertensive therapy (SHRU), (2) spontaneously hypertensive rats that received antihypertensive therapy (SHRT), (3) normotensive rats that received no antihypertensive therapy (NRU), and (4) normotensive rats that received antihypertensive therapy (NRT). At the beginning of the 3-month treatment period, each group comprised 12 rats. The mean weight of the normotensive rats was 150 ± 4 g. The age of both groups of rats at the beginning of the study was 12 weeks. During the period of study, blood pressure determinations were made periodically with an electrophysiomograph (model ESG-306, Narco Biosystems) attached to an E & M physiograph (DMP-4A, Narco Biosystems).

The rats were housed two to a cage. Untreated rats were allowed to drink distilled water ad libitum, but treated rats were only allowed to drink an antihypertensive drug solution ad libitum. This solution was prepared by dissolving the following agents in 1 liter of distilled water: (1) hydralazine HC1 (Appresoline, Ciba) (75 mg), (2) chlorothiazide (Diuril, Merck, Sharpe and Dohme) (1 g), and (3) reserpine (McKesson Laboratories) (1.4 mg). These regimens were continued for 3 months.

The aorta was isolated from each of the rats in the following manner. The rat was killed by a blow to the back of the head and exsanguinated by severing the jugular veins and the carotid arteries. The abdomen and the thorax were opened, the thoracic organs were dissected away, and the aorta was removed. The section of the aorta used was the portion from 1 cm above the diaphragm to 1 cm below the aortic arch. The excised aorta was immediately placed in cold (0-4°C) modified Tyrode’s solution saturated with 100% oxygen. An initial tension of 0.5 g was applied to the aorta and the aorta was cut on a spiral as described by Furchgott (19, 20) and Furchgott and Bursztyn (21). They formulated the following expression:

\[ \text{[Response]} = f(S) = f(e[DR]) = f(eR_T[D]) / \left(KDR + [D]\right), \]

where [Response] refers to the magnitude of the contractile response of the muscle, f refers to a real, single-valued function, S is the magnitude of the stimulus, e is the intrinsic efficacy, [DR] is the concentration of norepinephrine, R_T is the total concentration of receptor sites in the tissue, [D] is the concentration of norepinephrine, and K_DR is the dissociation constant for the norepinephrine-receptor complex. If e or R_T is reduced by some fraction, (1 - q), then the magnitude of the response will be reduced. If the original response can be reinstated by increasing the concentration of norepinephrine, then

\[ f(eR_T[D]) / \left(KDR + [D]\right) = f(eR_T[D]) / \left(KDR + [D']\right), \]

where [D'] is the concentration of norepinephrine that, in the experimental situation, elicits a response equal to that elicited by [D] under control conditions. Solving Eq. 2 for 1/[D] gives the following expression:

\[ 1 / \left[ D \right] = 1 / \left[ D' \right] + (1 - q) / q \left[ K_{DR} \right], \]

A plot of 1/[D] vs. 1/[D'] defines a straight line with a slope equal to (1/q) and an intercept equal to (1 - q)/(K_{DR}). Therefore,

\[ K_{DR} = \text{Slope} - 1 / \text{Intercept}. \]

Results

The blood pressure of the spontaneously hypertensive and the normotensive rats at the beginning of the study was 150 ± 7 (see) mm Hg and 110 ± 5 mm Hg, respectively. During the course of the study, the blood pressure of the SHRU group increased to 180 ± 9 mm Hg. The blood pressure of
Comparison of the norepinephrine dose-response curves shown in Figure 1 and 2 reveals that the tension produced by the aortic muscles from spontaneously hypertensive rats was significantly less than that produced by the aortic muscles from normotensive rats \((P < 0.001)\). This difference was evident for both treated and untreated spontaneously hypertensive and normotensive rats. In both cases, the magnitude of the response of aortic muscles from spontaneously hypertensive rats was approximately 50% of the response of aortic muscles from normotensive rats. However, the norepinephrine concentration required to induce a contraction equal to half of the maximum contractile response was not changed. These results indicate that an impairment of tension development in response to norepinephrine exists in muscle strips of spontaneously hypertensive rats relative to that in muscle strips of normotensive rats. This impairment persisted even when the high blood pressure was controlled.

The dose-response relationships between the norepinephrine concentration and the mechanical response of the aortic strips from the NRU, NRT, SHR, and SHRT groups are shown in Figures 1 and 2. The data in these figures are plotted as the negative logarithm of the molar concentration of norepinephrine in the bathing medium vs. the contractile response of the muscle (expressed as milligrams of tension development).

The NRU group increased to 135 ± 7 mm Hg. In contrast, the blood pressure of the SHRT group decreased to 113 ± 4 mm Hg and that of the NRT decreased to 109 ± 5 mm Hg during the first month of the 3-month treatment period; the blood pressures then remained at these levels throughout the rest of the treatment period. These results indicate that treatment of the rats was effective in controlling the hypertension. Thus, it was possible to determine \(K_{NR}\) in spontaneously hypertensive rats to which effective antihypertensive therapy had been administered.

The dose-response relationships between the norepinephrine concentration and the mechanical response of the aortic strips from the NRU, NRT, SHR, and SHRT groups are shown in Figures 1 and 2. The data in these figures are plotted as the negative logarithm of the molar concentration of norepinephrine in the bathing medium vs. the contractile response of the muscle (expressed as milligrams of tension development).
By interpolation, determinations were made in Figures 1 and 2 of the norepinephrine concentrations required to induce contractile responses of the muscle after exposure to phenoxybenzamine ([D']) that were equal to the contractile responses of the muscle induced by norepinephrine prior to exposure to phenoxybenzamine ([D]). The values of $K_{DR}$ obtained for the aortic muscles from the NRU and SHRU groups were $1.07 \times 10^{-7} \text{M}$ and $1.17 \times 10^{-7} \text{M}$, respectively (Figs. 3 and 4). These values of $K_{DR}$ for the aortic muscles from normotensive and spontaneously hypertensive rats that had received antihypertensive therapy are not significantly different. Moreover, the values of $K_{DR}$ for aortic muscles from rats that had received antihypertensive therapy are not significantly different from those for aortic muscles from rats that had received no antihypertensive therapy. These results indicate that antihypertensive therapy does not alter the affinity of the adrenergic receptor for norepinephrine.

**Discussion**

A basic question regarding the functional properties of blood vessels from normotensive and hypertensive animals is whether they exhibit differences in responsiveness to excitatory stimuli. Furthermore, if they do exhibit such differences, what is

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