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Physiological Variation in α-Adrenoceptor-Mediated Arterial Sensitivity: Relation to Agonist Affinity

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Vascular smooth muscle from different arteries of the rabbit varies in sensitivity to norepinephrine, even when factors known to contribute to this variation are excluded. Sensitivity to norepinephrine mediated through the α-adrenoceptor is linearly related to the agonist dissociation constant, but is not significantly related to receptor reserve. These results suggest that agonist affinity is the primary determinant of sensitivity to norepinephrine, at least in these arteries, and that this is a locally regulated characteristic which may account for regional sensitivity changes.

ARTERIAL SMOOTH MUSCLE EXHIBits both species and regional variation in its reactivity to norepinephrine (NE) (*1*). Even when factors known to contribute to this variation (for example, more than one type of receptor and local systems for sequestering or metabolizing NE) are excluded, remarkable variation of unknown basis remains (*2*). Differences in agonist affinity and receptor number (density) have been proposed as possible causes of variation in tissue sensitivity to drugs, but experimental evidence is lacking. In the rat vas deferens (*3*) and rabbit ovarian artery (*4*), the ability of various α-adrenoceptor agonists to evoke contraction was found to be related to the dissociation constants or affinity of these substances for the α-adrenoceptor. However, it is not known whether the varied sensitivities of different arteries to the same agonist are related to the affinity of that agonist for its receptors in these vessels. This possibility can be tested in the rabbit since the sensitivity to NE in a number of its arteries varies by more than two orders of magnitude.

We now report that the variation in the sensitivity to NE of 12 arteries of the rabbit from differing vascular regions can be correlated with the dissociation constant of NE for the α-adrenoceptor in these arteries. In all of these vessels the maximum response to NE was equal to the capacity of the tissue to contract, and the contractions that were used to determine NE sensitivities and dissociation constants were due to the action of NE on α_1-adrenoceptors (see below).

Arterial ring segments (3 to 3.5 mm) were mounted in vitro in Krebs solution, at 37°C, equilibrated with 95% O_2 and 5% CO_2. Standard pharmacological procedures were used for measurement of isometric changes in wall tension (*5*). Segments were stretched to their optimum rest tension, which had been determined in preliminary experiments with each vessel type. Desmethylimipramine ($1 \times 10^{-7} M$), deoxycorticosterone acetate ($1 \times 10^{-5} M$), and propranolol ($1 \times 10^{-6} M$) were added to the bath solution to block neuronal and extraneuronal uptake of NE and to block β-adrenoceptors, respectively. These are recognized factors that influence the response of the blood vessel to NE (*6*). Norepinephrine was added cumulatively to generate data for a dose-response curve from which the agonist sensitivity ($pD_2 = -\log EC_{50}$, where EC_{50} is the median effective concentration) was obtained. After the maximum contraction in response to NE had been recorded, the addition of serotonin ($1 \times 10^{-3} M$) or histamine ($1 \times 10^{-3} M$) did not cause further contraction of any artery. EC_{50}'s were obtained from contractile responses between 20% and 80% of maximum by linear regression analysis of probit response–log concentration data. One determination was made from each artery. A minimum of five of each type of artery was studied, each one from a different rabbit. Some experiments with each type of artery were carried out after removal of the endothelium by rubbing. The effectiveness of this procedure was confirmed by an absence of dilation in response to acetylcholine ($1 \times 10^{-8} M$ to $1 \times 10^{-6} M$) and by microscopic examination after $AgNO_3$ processing and en face examination (*7*). The agonist dissociation constant (K_A) was determined according to the method of Furchgott and Bursztyn (*8*). After control responses were obtained, the tissues were treated with dibenamine ($3 \times 10^{-7} M$ to $1 \times 10^{-6} M$) for 15 minutes and washed for another 30 minutes before NE was added again. Equieffective concentrations of NE before (A) and after (A') dibenamine treatment were obtained. The slope and y-intercept of the regression line of $1/A$ against $1/A'$ were used to calculate K_A (slope − 1/intercept). The contractions in

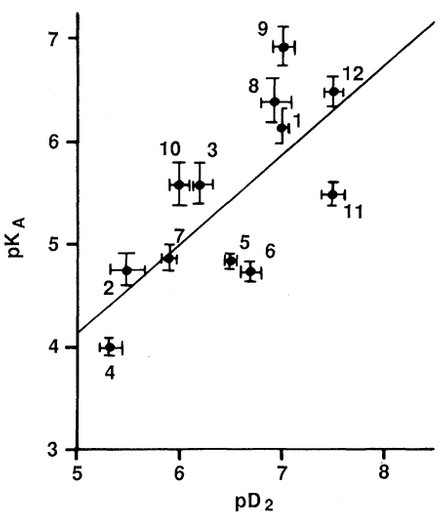

Fig. 1. Relation between the norepinephrine dissociation constant for the α-adrenoceptor (pK_A) and sensitivity (pD_2) of 12 arteries from the rabbit. The equation of the regression line is $y = 0.88x - 0.23$ and the SEM of the slope is 0.26 ($P < 0.01$). Brackets show the standard errors of means of at least five determinations, each one on an artery from a different rabbit: 1, abdominal aorta; 2, superior mesenteric artery; 3, renal artery; 4, ovarian artery; 5, common iliac artery; 6, external iliac artery; 7, internal iliac artery; 8, large pulmonary artery; 9, medium pulmonary artery; 10, basilar artery; 11, ear artery, and 12, thoracic aorta.

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response to NE that were used in the calculation of K_A were invariably blocked completely by prazosin ($10^{-7}M$).

There was a positive correlation between pD_2 and the agonist dissociation constant (pK_A) for 12 types of rabbit arteries (slope ± SEM = 0.88 ± 0.26; $P < 0.01$). This slope was not statistically significantly different from unity (Fig. 1). Only with the superior mesenteric artery did endothelium removal increase the pD_2, and then by a factor of 3.5. For this artery, the value after endothelium removal was used. The percentage of available receptors occupied influences tissue sensitivity (*1, 9*). In a blood vessel the smaller the percentage of available receptors occupied, the greater the sensitivity. When the results from three arteries—the ear artery and common and external iliac arteries—with a high receptor reserve [defined as antilog($-\log EC_{50} - pK_A$)] were excluded, the correlation between pD_2 and pK_A was higher, and its slope was not statistically significantly different from unity. There was no statistically significant correlation between receptor reserve and pD_2 (slope ± SEM = 15.8 ± 14.3; $P > 0.05$). Thus, at least in these arteries, although there was some suggestion that receptor occupancy influences tissue sensitivity, the data were consistent with the conclusion that the dominant factor is the agonist dissociation constant.

Variation in K_A values for NE in various in vitro preparations—for example, $1.3 \times 10^{-7}M$ in the rabbit aorta (*10*) and $6.3 \times 10^{-6}M$ in the rat vas deferens (*3*)—has been taken as an indication of heterogeneity of α_1-adrenoceptors. However, our demonstration of a relation between agonist potency and dissociation constant of the same full agonist in anatomically different but structurally similar systems, indicates that the α_1-adrenoceptors in the different arteries are similar, but that they are present in the smooth muscle cells in different affinity states. This variation probably is determined by endogenous factors in the receptor microenvironment.

Recent studies of α_1-adrenoceptor ligand binding associated with Ca^{2+} efflux in cultured vascular smooth muscle cells dispersed from rabbit aorta suggest a population of receptors that exists in both high- and low-affinity states. With this assumption, 40% of the receptors in the rabbit aorta are in the high-affinity state (*11*). However, the ratio of affinities of the two states is only 86. This is small in comparison to differences of more than 500 between receptor systems represented by the 12 arteries in this series. Thus, unless there are additional influences that increase the range between the two states, this explanation would not account for the variation we have found. In the series of arteries that we studied, intracellular regulation of α-adrenoceptor affinity could be responsible for this diversity.

The NE sensitivities of a number of rabbit regional arteries—ear, pulmonary, and mesenteric—are markedly different, and these differences are maintained as they branch (*1*). Thus it seems that α-adrenoceptor affinity is a locally regulated characteristic of vascular smooth muscle which can account for regional differences in sensitivity to NE. As in some instances, patterns of regional differences can be related to embryological development (*1*), such differences in these regulating systems may be established early in development.

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Common Mechanism of Chromosome Inversion in B- And T-Cell Tumors: Relevance to Lymphoid Development

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An inversion of chromosome 14 present in the tumor cells of a patient with childhood acute lymphoblastic leukemia of B-cell lineage was shown to be the result of a site-specific recombination event between an immunoglobulin heavy-chain variable gene and the joining segment of a T-cell receptor α chain. This rearrangement resulted in the formation of a hybrid gene, part immunoglobulin and part T-cell receptor. Furthermore, this hybrid gene was transcribed into messenger RNA with a completely open reading frame. Thus, two loci felt to be normally activated at distinct and disparate points in lymphocyte development were unified and expressed in this tumor.

ASSOCIATION OF A SPECIFIC CHROMOSOMAL abnormality with a specific tumor type is well established and may reflect mechanisms of oncogenesis peculiar to that tumor (*1*). Alternatively it may be that these associations reflect the particular differentiated state of the malignant cell, consistent with the model that rearrangements occur only within chromatin in an "active" configuration (*2*). We and others recently reported the molecular analysis of a chromosomal abnormality in the cell line SUP-T1 that was derived from a pediatric patient with T-cell lymphoma (*3*). The SUP-T1 cell line contained a paracentric inversion of chromosome 14, inv(14)(q11.2q32.3), which is commonly seen in T-cell malignancy (*4, 5*). We demonstrated that this abnormal rearrangement was the result of site-specific recombination that could directly produce the inversion by uniting a T-cell receptor α-chain joining segment (TCR J_α) from band q11.2 with the immunoglobulin heavy-chain variable gene (Ig V_H) from band q32.3. This finding demonstrated that not only are the Ig and TCR loci similar in genomic structure but on occasion their component parts can be shuffled to produce a transcriptionally active

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