lung [25], the drug-metabolizing enzymes probably reside in either the type II alveolar cells or the Clara cells, since these have abundant endoplasmic reticulum [26]. Following cortisol administration, the type II cells mature earlier, and this correlates with increased lung phospholipid content [7]. In contrast, Wang *et al.* [6] report that at the same gestational age the Clara cells in fetal lungs are not well developed, do not respond to glucocorticoid treatment and are less likely to be involved in secreting surface-active compounds. It appears that, in the 27-day fetal rabbit, cortisol acts to induce enzymes involved in lung phospholipid synthesis specifically in type II cells without influencing the drug-metabolizing enzymes. Thus, the enzymes responsible for metabolizing drugs may not reside in the type II cells at all, or the appearance of these enzymes in fetal rabbits is under some other control mechanism. Localization of drug-metabolizing enzymes within Clara cells, however, cannot be ruled out on the evidence presently available. In this regard, it is interesting that Clara cells have been implicated recently as the site of oxidative metabolism in the lung of 4-ipomeanol [27]. Whether the pulmonary drug-metabolizing capacity is confined to the Clara cells and whether these cells are capable of metabolizing other xenobiotics have yet to be determined. Experiments in progress in our laboratory are aimed at determining the ability of isolated pulmonary type II cells to metabolize drugs.

The results presented in this communication indicate strongly that, although cortisol plays a role in accelerating lung maturation in the rabbit fetus, it does not induce fetal pulmonary or hepatic drug-metabolizing enzymes in this species.

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*Laboratory of Toxicology, National Cancer Institute, National Institutes of Health, Bethseda, MD* 20014, *U.S.A.*

ZAHID H. SIDDIK
BRANIMIR I. SIKIC
ROGER DREW
EDWARD G. MIMNAUGH
CHARLES L. LITTERST
THEODORE E. GRAM

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## Effects of prazosin on cyclic nucleotide content and blood pressure of the spontaneously hypertensive rat

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The involvement of abnormal levels of cyclic nucleotides in the pathophysiology of hypertension has been suggested by several workers [1–7]. This idea fits the concept that intracellular levels of cyclic nucleotides are one of the determinants of vascular smooth muscle tone [8]. Prazosin, a potent antihypertensive agent, was developed as an inhibitor *in vitro* of cyclic 3′,5′-nucleotide phosphodiesterase (EC 3.1.4.17) (PDE) [9]. It has been proposed that this drug leads to a reduction of blood pressure by raising vascular smooth muscle intracellular cyclic 3′,5′-adenosine monophosphate (cyclic AMP). The development of prazosin has recently been cited as an example of rational drug development [10]. However, the effects of this drug on cyclic nucleotide levels *in situ* have not been reported. We have studied the effects of prazosin on cyclic nucleotide levels in the aorta and aortic smooth cells in tissue culture from the

spontaneously hypertensive rat (SHR). This strain of rat was inbred by Okamoto and Aoki [11] to be hypertensive. The pathophysiology found in this animal is thought to resemble that of human essential hypertension [12]. Our studies with smooth muscles support the proposal of Oates *et al.* [13] that prazosin lowers blood pressure via α-adrenergic blockade in the vasculature, rather than as an inhibitor of phosphodiesterase. Thus, it appears that prazosin does not act directly on smooth muscle to produce relaxation.

Spontaneously hypertensive rats were purchased from Charles River, Lakeview Breeding Laboratories, Wilmington, MA. Blood pressure was determined using a Narco Biosystems sphygmomanometer for indirect measurement of tail artery pressure. Male rats, paired for age, weighing 200–300 g, were used. Prazosin was a gift from the Pfizer Corp, Groton, CT.

Aortas were removed from rats and prepared for the determination of cyclic nucleotides as described previously [6]. Cyclic AMP content was determined by the binding assay described by Gilman [14], as modified by Tovey *et al.* [15]. Cyclic 3′,5′-guanosine monophosphate (cyclic GMP) content was determined using radioimmunoassay kits obtained from New England Nuclear Corp., Boston, MA for the detection of cyclic GMP after acetylation with acetic anhydride [16].

Smooth muscle cells derived from the aortas of SHR rats were grown in tissue culture following the procedures previously described [6]. For drug studies, 150 22 mm plates were seeded with 4 $10^5$ cells. After 8 days the medium was removed and the cells were washed with phosphate-buffered saline (PBS). The drug was added at the indicated dose in PBS and the cultures were allowed to incubate at 37° in an atmosphere of 5% $CO_2$–95% air for 15 min. At that time the drug–PBS mixture was removed and the plates were covered with 10 ml of 5% trichloroacetic acid. The cells were scraped from the plates with a rubber policeman and homogenized with a polytron. After centrifugation the supernatant fraction was prepared for cyclic nucleotide assays as described previously [6]. The pellet was dissolved in 5 ml of 5 N NaOH and assayed for protein content by the microbiuret procedure [17].

The data are reported as the mean ± S.E. Comparisons were made using Student's *t*-test.

If the level of cyclic nucleotides was one of the determinants of the blood pressure in the SHR rat, one would expect that prazosin, an agent which inhibits PDE *in vitro* and is a potent hypotensive drug, would raise cyclic AMP levels in the aorta. Prazosin acts extremely rapidly. After an 0.5 mg/kg (i.p.) injection the blood pressure of an SHR rat begins to drop within the first 2 min (data not shown). To determine if prazosin affects cyclic AMP content before a pharmacologic response could be seen, animals were decapitated 1 min after an 0.5 mg/kg (i.p.) injection of prazosin, and the cyclic AMP content was determined. At this time the pharmacologic response to the drug was just beginning. Due to this rapid action of prazosin and the short time before the pharmacologic response could be seen, blood pressure was not determined in this experiment. The data presented in Table 1 show that prazosin, at this time, had no statistically significant effect on the cyclic AMP content of either the aorta or heart.

Table 1. Effect of prazosin on cyclic AMP levels in the SHR rat aorta and heart*

| Organ | Cyclic AMP[2] (pmoles/mg wet weight) Control | Prazosin |
|---|---|---|
| Aorta | 1.97 ± 0.16 (5) | 2.36 ± 0.36 (5) |
| Heart | 0.41 ± 0.04 (5) | 0.54 ± 0.07 (5) |

* Prazosin (0.5 mg/kg, i.p.) was injected 1 min before killing the animal. The numbers in parentheses indicate the number of observations.

To determine if prazosin affected cyclic nucleotide content at the time of the maximal hypotensive effect, both cyclic nucleotide content and blood pressure were measured 1 hr after treatment of rats with 0.5 mg/kg (i.p.) of prazosin. Table 2 shows the effects of prazosin on blood pressure and cyclic nucleotide content of the aorta and heart. While prazosin markedly lowered blood pressure (from 152 ± 2 to 106 + 3 mm Hg), neither the cyclic AMP nor the cyclic GMP content of the aorta or heart was changed. The relatively low cyclic GMP levels found in the heart are similar to those reported by Ramanathan and Shibata [18] for SHR rat hearts of similar age. As a positive control to determine if changes in cyclic AMP could be detected, several rats were injected with isoproterenol and an approximate doubling of the aortic cyclic AMP content was seen (data not shown).

The aorta contains numerous cell types and it is possible that the cyclic nucleotides measured were not from the smooth muscle cells. To eliminate this possibility, aortic smooth muscle cells were grown in tissue culture and the effect of prazosin on the intracellular content of cyclic nucleotides was determined. As shown in Fig. 1, prazosin alone did not raise the cyclic AMP or cyclic GMP content of these cells. 3-Isobutyl-1-methylxanthine (MIX), a potent PDE inhibitor, also did not affect the cyclic nucleotide content. However, as expected of a PDE inhibitor, MIX did potentiate the increase in cyclic AMP content seen after isoproterenol, a β-adrenergic agonist. With regard to the isoproterenol-induced increase in cyclic GMP, this drug has been reported recently to stimulate broken cell cytosolic guanylate cyclase activity [19]. Prazosin, instead of potentiating the effects of isoproterenol, as would be expected of a PDE inhibitor, reduced the isoproterenol effect on both cyclic AMP and cyclic GMP content.

These data do not support the hypothesis that prazosin acts as a PDE inhibitor *in vivo* to produce smooth muscle relaxation. The reduction in the isoproterenol-induced increase in cyclic nucleotides produced by prazosin in the tissue culture experiments is intriguing. We have no explanation for this observation. The data suggest that prazosin may affect the drug sensitivity of adenylate and guanylate cyclase or inhibit their enzymatic activity. Further study is clearly warranted.

Table 2. Effect of prazosin on cyclic nucleotide levels in the SHR rat aorta and heart*

| | Organ | Control | Prazosin |
|---|---|---|---|
| Cyclic AMP (pmoles/mg wet weight) | Aorta | 0.68 ± 0.11 (5) | 0.80 ± 0.24 (5) |
| | Heart | 0.60 ± 0.05 (5) | 0.68 ± 0.06 (5) |
| Cyclic GMP (pmoles/mg wet weight) | Aorta | 0.045 ± 0.006 (5) | 0.045 ± 0.006 (5) |
| | Heart | 0.006 ± 0.001 (4) | 0.006 ± 0.001 (4) |
| Blood pressure (mm Hg) | | 151 ± 4†(5) | 105 ± 4†(5) |

* Prazosin (0.5 mg/kg, i.p.) was injected 1 hr before killing the animal. The number in parentheses indicate the number of observations.

† $P < 0.05$.

We did not study the entire vascular system. We measured cyclic nucleotide levels only in aorta, heart, and aorta smooth muscle cells in tissue culture. While there is abundant evidence that essential hypertension is a consequence of elevated peripheral vascular resistance, the data reported here using the intact rat aorta, heart, and aorta smooth muscle cells in culture minimized the danger that drug effects at the smaller peripheral vessels might be important and thus be missed. The effects of prazosin on the smooth muscle cells in tissue culture and on the heart should reflect the mode of action of prazosin on any vascular muscle cells. The aorta, as well as smaller vessels, and the heart of the SHR rat have been reported to contain reduced levels of cyclic AMP [4–7]. Cyclic AMP levels in aorta and heart were determined at 1 min and at 1 hr after an i.p. injection of prazosin. At these times the hypotensive effect was either just beginning or maximal. The data suggest that a transient change in cyclic AMP content does not occur early in the pharmacological response and act as a signal for the hypotensive effect of the drug.

The roles of cyclic AMP and cyclic GMP in controlling smooth muscle tone are unclear in the light of recent work [20–23]. It now appears that cyclic GMP may play a role in smooth muscle relaxation [23] rather than being involved in smooth muscle contraction. The results presented here with regard to cyclic AMP and cyclic GMP do not support the concept that one of the determinants of blood pressure is the level of cyclic nucleotides found in the vascular system.

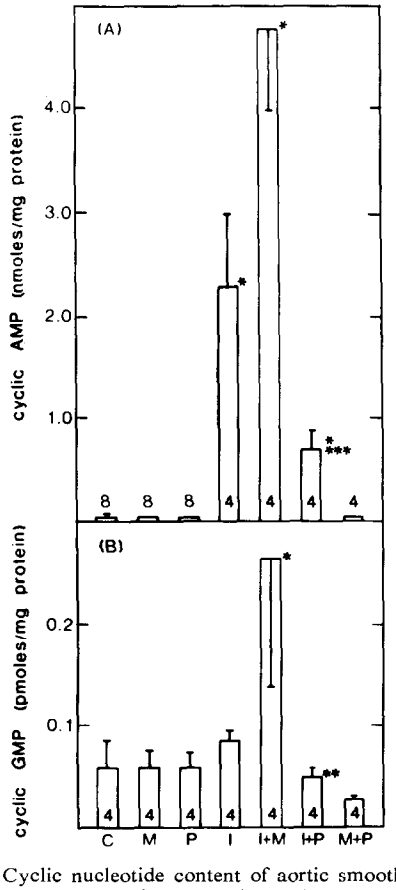


Fig. 1. Cyclic nucleotide content of aortic smooth muscle cells in tissue culture after a 15-min incubation with various drugs. Key: C = control; M = 3-isobutyl-1-methylxanthine ($10^{-4}$ M) (MIX); P = prazosin ($10^{-4}$ M); I = isoproterenol ($2 \times 10^{-6}$ M); a single asterisk (*) = $P < 0.05$ when compared to control; a double asterisk (**) = $P < 0.05$ when compared to isoproterenol; and a triple asterisk (***) = $P < 0.05$ when compared to isoproterenol + MIX.

These data do support the suggestion by Oates *et al.* [13] that prazosin exerts its hypotensive effect by blocking α-adrenergic nerve impulses rather than by acting directly on the smooth muscle. The data in this report also point out the pitfalls that are still present in "rational drug development." Prazosin was conceived to be a combination of the important structural features of two PDE inhibitors, papaverine and theophylline [10], and is a potent PDE inhibitor *in vitro* [9]. However, the data presented in this report do not support PDE inhibition as a mode of action of the drug in smooth muscle relaxation.

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*Division of Molecular and Cellular Biology,*
*National Jewish Hospital and Research Center,*
*Denver, CO 80206, U.S.A.*

HOWARD SANDS
RICHARD JORGENSEN

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