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In Vitro Drug Absorption Models. II. Salicylate, Cefoxitin, α-Methyldopa and Theophylline Uptake in Cells and Rings: Correlation with *In Vivo* Bioavailability

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Abstract: Isolated mucosal cells and everted intestinal rings have been examined as potential *in vitro* models for intestinal drug absorption. The uptake of salicylate, cefoxitin, α-methyldopa and theophylline was characterized on the basis of time, concentration and temperature dependence and compared to *in vivo* drug absorption. Theophylline was well absorbed in all systems. Biochemical studies supported a passive transport mechanism, although a significant temperature dependence was observed. Salicylate, cefoxitin and α-methyldopa demonstrated time- and concentration-dependent absorption. The uptake of α-methyldopa was temperature-dependent in both the isolated cell and ring studies. With all drugs, cellular uptake exhibited greater variability than drug accumulation in rings. A comparison of *in vitro* and *in vivo* absorption demonstrated a good correlation between the data from *in vivo* studies and intestinal rings. Cellular drug uptake did not completely mimic that observed *in vivo*. On the basis of technical aspects of preparation, reproducibility of results, and correlation with *in vivo* drug bioavailability, everted intestinal rings were judged to be the best *in vitro* model for intestinal drug absorption.

Drug absorption studies have typically been performed using whole animal models. Serum, plasma or urine drug levels are monitored following administration of an experimental dosage form, and conclusions based on pharmacokinetic analysis are formed. While this approach provides valuable information and is the ultimate test for drug absorption, there are certain disadvantages or restrictions with this method. Experiments are costly and time consuming, and a detailed analysis of drug interaction with the mucosal barrier membrane is difficult. Experimental variables are not easily controlled since the biological system is constantly adjusting to maintain homeostasis.

In attempts to study nutrient, ion or drug transport on a more detailed level, various *in situ* and *in vitro* models have been employed. *In situ* intestinal perfusions (1–3), isolated segments (4), everted sacs (5), intestinal rings (4), isolated cell suspensions (6–8) and membrane vesicles (9) have all been employed with varying degrees of success. Often, these systems have been investigated independently, without suitable comparisons between models or without comparison to known *in vivo* transport properties of the compounds under investigation.

In a previous report (10), we described the isolation and characterization of rat brush border membrane vesicles, mucosal cells and everted intestinal rings. Based on structural and functional integrity, reproducibility of results and demonstrable drug transport, everted intestinal rings and isolated mucosal cells were chosen for further evaluation as potential *in vitro* models for oral drug absorption. The transport of cefoxitin, salicylate, α-methyldopa and theophylline were examined in these *in vitro* systems and compared to *in vivo* absorption. The four model drugs were chosen to provide a series of test compounds with varying physical properties and anticipated differences in intestinal absorption profiles. Projections as to the utility of mucosal cells and isolated rings as suitable models for studying intestinal drug absorption are presented. The

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Table I. Characterization of Salicylate Uptake into Mucosal Cells and Intestinal Rings

	Salicylate Uptake (mean ± S.E.)	
	Cells (μg/mg protein)	Rings (μg/mg wet wt.)
Concentration (mg/ml)		
1.0	3.0 ± 0.1[a]	–[b]
5.0	11.9 ± 0.6	1.0 ± 0.04
10.0	26.5 ± 1.0	1.6 ± 0.05
20.0	57.1 ± 1.2	4.0 ± 0.18
Temperature (°C)		
37	9.0 ± 2.6[c]	2.0 ± 0.02[d]
25	9.0 ± 1.8	–
4	15.3 ± 1.5	1.8 ± 0.05

Specific incubation conditions for each set of values are denoted by superscript members. The results are based on 3–12 determinations.
Incubation conditions:
[a]2 min incubation, 37°C, n = 12
[b]5 min incubation, 37°C, n = 3–5
[c]2 min incubation, [Salicylate] = 10 mg/ml, n = 3
[d]2 min incubation, [Salicylate] = 20 mg/ml, n = 3

Table II. Characterization of Cefoxitin Uptake into Mucosal Cells and Intestinal Rings

	Cefoxitin Uptake (mean ± S.E.)	
	Cells (μg/mg protein)	Rings (μg/mg wet wt.)
Time (min)		
0	8.1 ± 0.4[a]	0.3 ± 0.02[b]
2	6.8 ± 0.3	0.9 ± 0.05
5	–	1.6 ± 0.03
10	5.6 ± 0.8	1.7 ± 0.20
20	–	1.2 ± 0.01
Concentration (mg/ml)		
1	–[c]	0.03 ± 0.01[d]
5	6.8 ± 0.3	0.07 ± 0.02
10	9.6 ± 2.5	0.18 ± 0.03
20	–	0.39 ± 0.03
Temperature (°C)		
37	1.5 ± 1.7[e]	0.96 ± 0.05[e]
25	1.2 ± 0.7	–
4	3.2 ± 0.6	0.74 ± 0.05

Specific incubation conditions for each set of values are denoted by superscript numbers. The results are based on 3–5 determinations.
Incubation conditions:
[a][Cefoxitin] = 5 mg/ml, 37°C
[b][Cefoxitin] = 10 mg/ml, 37°C
[c]2 min incubation, 37°C
[d]3 min incubation, 37°C
[e][Cefoxitin] = 10 mg/ml, 2 min incubation

potential of the *in vitro* models for routine screening and detailed mechanistic studies is discussed.

Experimental

Animals

Male Sprague-Dawley rats (200–250 g) were used in all experiments. Animals were fasted for 18 h prior to all studies. Water was provided ad libitum.

Tissue Preparation

Isolated mucosal cells and everted intestinal rings were prepared as previously described (10). Isolated cells were used only if the cell viability, as determined by Trypan blue dye exclusion, exceeded 80 %. Cells and rings were stored in buffer or Eagle's culture medium at 0–4°C prior to *in vitro* incubations. Continous aeration with 95 % O_2/5 % CO_2 was provided.

Drug Uptake Studies

Cells were suspended in Eagle culture medium and incubated at 37°C or 4°C under O_2/CO_2. Ascorbic acid (0.1 %) was included in the culture medium to prevent α-methyldopa oxidation. Following incubation in the presence of drug, the cells were isolated by centrifugation through a layer of 12 % sucrose containing 1.0 % bovine serum albumin. The cell pellet was then resuspended in the appropriate medium for extraction and subsequent HPLC assay of drug content.

Everted intestinal rings were incubated at 37°C with O_2/CO_2 aeration in the presence of drug and according to specific experimental conditions. Ascorbic acid (0.1 %) was included in the incubation solutions to prevent α-methyldopa oxidation. At the end of the incubation period, the rings were rinsed in buffer, blotted dry with Whatman No. 1 filter paper, and placed in 1 ml of 1 N hydrochloric or perchloric acid. After 12 h, aliquots of the resulting tissue extracts were removed and frozen for subsequent HPLC assay. Results were corrected for extracellular space as previously described (10).

In Tables I–IV, each grouping of data was collected from the same preparation of cells or rings. Because of limited availability of cells or rings from each animal, different preparations were occasionally used for the various experiments reported in each Table.

Protein Assay

Protein was measured by the method of Lowry and coworkers (11) with bovine serum albumin as the standard.

Drug Assays

Cefoxitin (12), salicylate (10), theophylline (13, 14) and α-methyldopa (15) were assayed by high performance liquid chromatography.

The cefoxitin assay (12) employed a Brownlee 10 μM RP-8 column with an eluting solvent of 100 ml pH 6.86 Fisher Gram-Pak buffer, 110 ml methanol and 10 ml acetonitrile diluted to 1000 ml with water. A flow rate of 3.0 ml/min was maintained, with detection by absorbance at 254 nm with sodium cefmetazole as an internal standard. The detection limit was 0.1 μg/ml with a coefficient of variation of 5.9 % at 6.25 μg/ml.

Salicylate was assayed as previously described (10) with detection at 303 nm. A Brownlee RP-18 column was used with an eluting solvent of 18 % acetonitrile, 0.04 % ammonium and 0.4 % formic acid. The detection limit was 0.1 μg/ml with a coefficient of variation of 5.6 % at 1.0 μg/ml.

The theophylline assay (13, 14) employed a Brownlee RP-18 column with an eluting solvent of 7 % acetonitrile and 10 mM sodium acetate (pH 4.0). Detection was at 280 nm with a flow rate of 3.0 ml/min. Caffeine was used as an internal standard. The detection limit was 0.3 μg/ml with a coefficient of variation of 6.8 % at 1.5 μg/ml.

Separation of α-methyldopa in the HPLC analysis (15) was effected with a primary 4.6 × 10 mm Brownlee RP-18 column and a secondary 2.1 × 10 ml Brownlee RP-18 column with a

Table III. Characterization of α-Methyldopa Uptake into Mucosal Cells and Intestinal Rings

	α-Methyldopa Uptake (mean ± S.E.)	
	Cells (μg/mg protein)	Rings (μg/mg wet wt.)
Time (min)		
0	15.7 ± 2.9[a]	–[b]
2	24.7 ± 3.8	0.06 ± 0.01
5	–	0.10 ± 0.001
10	36.3 ± 9.4	0.12 ± 0.02
20	–	0.16 ± 0.02
Concentration (mg/ml)		
0.2	–[c]	0.03 ± 0.003[d]
0.5	1.7 ± 0.30	–
1.0	0.6 ± 0.07	0.15 ± 0.02
2.0	–	0.25 ± 0.01
4.0	–	0.26 ± 0.01
5.0	3.2 ± 0.2	–
10.0	–	0.31 ± 0.02
Temperature (°C)		
37	3.2 ± 0.2[e]	0.27 ± 0.02[f]
25	–	0.18 ± 0.01
4	0.0	0.12 ± 0.01

Specific incubation conditions for each set of values are denoted by superscript numbers. The results are based on 3–5 determinations.
Incubation conditions:
[a][α-Methyldopa] = 5 mg/ml, 37°C
[b][α-Methyldopa] = 1 mg/ml, 37°C
[c]2 min incubation, 37°C
[d]5 min incubation, 37°C
[e][α-Methyldopa] = 5 mg/ml, 2 min incubation
[f][α-Methyldopa] = 1 mg/ml, 5 min incubation

Table IV. Characterization of Theophylline Uptake into Mucosal Cells and Intestinal Rings

	Theophylline Uptake (mean ± S.E.)	
	Cells (μg/mg protein)	Rings (μg/mg wet wt.)
Time (min)		
0	–[a]	0.06 ± 0.004[b]
1	–	0.46 ± 0.03
2	49.4 ± 2.4	0.68 ± 0.04
5	58.7 ± 2.1	1.31 ± 0.11
10	67.8 ± 2.5	1.85 ± 0.08
20	–	2.24 ± 0.17
Concentration (mg/ml)		
1	3.8 ± 0.3[c]	0.25 ± 0.09[c]
5	22.8 ± 1.0	1.00 ± 0.16
10	53.8 ± 3.0	2.39 ± 0.99
Temperature (°C)		
37	53.8 ± 3.0[d]	0.68 ± 0.04[e]
25	–	–
4	57.8 ± 4.4	0.16 ± 0.02

Specific incubation conditions for each set of values are denoted by superscript numbers. The results are based on 3–6 determinations.
Incubation conditions:
[a][Theophylline] = 10 mg/ml, 37°C
[b][Theophylline] = 2.5 mg/ml, 37°C
[c]2 min incubation, 37°C
[d][Theophylline] = 10 mg/ml, 2 min incubation
[e][Theophylline] = 2.5 mg/ml, 2 min incubation

mobile phase of 5.0 % acetonitrile and 0.1 % phosphoric acid (pH 2.15). A flow rate of 1.0 ml/min was maintained, and the eluent was monitored with an ESA Coulocomb 5100 A electrochemical detector at −0.05 V and +0.10 V. Stability of α-methyldopa was verified in the analytical procedures by parallel processing of standard solutions. The detection limit was 0.1 μg/ml with a coefficient of variation of 3.2 % at 0.5 μg/ml.

In Vivo Drug Absorption

Each animal received either intravenous (4 mg/kg) or intraduodenal (dose shown in Table V) injections of cefoxitin, α-methyldopa, salicylate and theophylline. Ascorbic acid (0.1 %) was included in the α-methyldopa solutions. Blood samples were collected at 10, 20, 30, 60 and 90 min post-dosing, and the areas under the plasma drug concentration versus time profiles (AUC) were calculated by trapezoid summation with truncation at the last plasma time point. Per cent bioavailability was determined by comparing the intravenous and intraduodenal AUC on a dose adjusted basis.

Results

The characterization of salicylate, cefoxitin, α-methyldopa and theophylline uptake into isolated mucosal cells and everted intestinal rings is summarized in Tables I, II, III and IV. Time, concentration and temperature dependence was examined with each drug in both *in vitro* systems.

Salicylate accumulation (Table I), previously shown to be time-dependent in both isolated cells and rings (10), was also characterized by non-saturable concentration dependence between 1 mg/ml and 20 mg/ml for cells, and 5 mg/ml to 20 mg/ml for rings. Salicylate uptake did not decrease significantly when the incubations were performed at 4°C instead of 37°C.

Cefoxitin accumulation (Table II) in cells and rings was somewhat erratic, especially in the isolated cell system. Cellular uptake of cefoxitin actually decreased with increasing incubation times at 37°C. Only two concentrations of cefoxitin were tested with cells, and the results demonstrated slightly increased, but more variable, absorption at the higher concentration (10 mg/ml). Cefoxitin uptake into cells was not decreased at reduced incubation temperatures (4°C). With intestinal rings, cefoxitin accumulation was time-dependent between 0 and 5 minutes of incubation at 37°C. From 5 to 20 min of incubation, the level of cefoxitin in the rings either remained constant or decreased. Concentration-dependence between 1 mg/ml and 20 mg/ml was non-saturable, and only a slight temperature-dependence (−23 %, $p < 0.05$) was observed.

The absorption characteristics of α-methyldopa into isolated cells and rings (Table III) was significantly different than that observed with salicylate or cefoxitin. Time-dependent uptake was seen with both the cell and ring systems. The concentration-dependence was erratic with isolated cells, but it was apparently saturable with intestinal rings. The most striking feature of α-methyldopa uptake was the temperature-dependence. Accumulation of α-methyldopa was completely inhibited at 4°C in isolated cells and reduced by 56 % ($p < 0.01$) in the rings. Even at 25°C, α-methyldopa uptake was reduced 24 % ($p < 0.05$) when compared to incubations at 37°C.

Theophylline uptake (Table IV) was time- and concentration-dependent (non-saturable) in both systems. Cell studies did not indicate any temperature-dependence for theophylline uptake. In contrast, theophylline accumulation was reduced by

77 % ($p < 0.01$) when intestinal rings were incubated at 4°C instead of 37°C.

In order to further confirm the temperature-dependence observed for theophylline uptake into intestinal rings, a time-dependence study at 37°C and 4°C was performed. As can be seen in Figure 1, a significant temperature-dependence was observed throughout the 20 min incubation period.

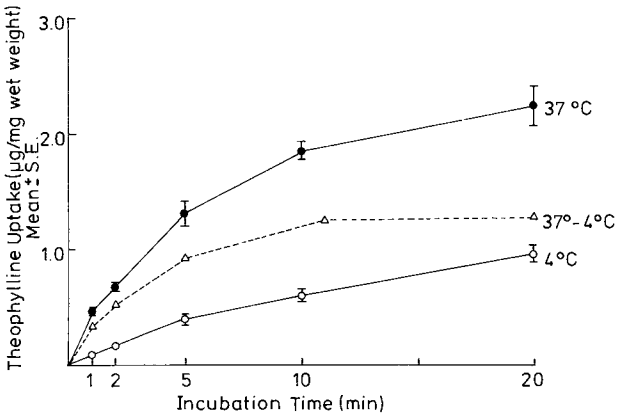

Fig. 1 Time and temperature dependence of theophylline uptake into everted intestinal rings. Rings were incubated at 37°C or 4°C in the presence of 2.5 mg/ml theophylline for varying lengths of time (1–20 min). Each point is the mean ± S.E. for n = 6 determinations. The dashed line (37° – 4°C) represents the difference between the experimental values measured at 37°C and 4°C.

Theophylline transport into intestinal rings was further examined under a variety of experimental conditions. Pre-treatment and incubation of the rings with 5 µM ouabain, 5 µM 2,4-dinitrophenol, or in sodium-free medium did not reduce theophylline accumulation (data not shown). Uptake was slightly reduced in the presence of equimolar amounts of adenine (−28 %), guanine (−31 %), inosine (−33 %) and xanthine (−38 %), although the results were not statistically different at the $p < 0.05$ level. Dipyridamole, an inhibitor of purine transport (16), was ineffective at 7 mM in inhibiting theophylline accumulation by intestinal rings.

The *in vivo* absorption of cefoxitin, α-methyldopa, salicylate and theophylline is shown in Table V. Theophylline was well absorbed, while cefoxitin absorption was essentially negligible. Intermediate levels of drug absorption were observed for α-methyldopa and salicylate.

Table V. *In Vivo* Absorption of Cefoxitin, α-Methyldopa, Salicylate and Theophylline in Rats Following Duodenal Administration

Drug	Oral Dose (mg/kg)	% Bioavailability (mean ± S.E.)	n
Cefoxitin	10	2 ± 0.8	4
α-Methyldopa	10	19 ± 5.7	3
Salicylate	16	22 ± 0.7	16
Theophylline	10	75 ± 2.1	6

Each animal received either intravenous or intraduodenal drug administration at the indicated dose (aqueous vehicle under urethane anesthesia). Blood samples were collected for 2 h post-dosing. Areas under the plasma drug concentration versus time profiles were calculated. Per cent bioavailability was determined by comparing the intravenous and intraduodenal areas on a dose adjusted basis.

In order to qualitatively compare the observed *in vivo* absorption with drug uptake by mucosal cells and rings, the absorption data were normalized and are shown in Figure 2. Since theophylline absorption was the highest in all three systems, the per cent theophylline absorption was set at 100. Salicylate, cefoxitin and α-methyldopa absorption was then normalized relative to theophylline. With both cells and rings, salicylate was absorbed to a greater extent than cefoxitin and α-methyldopa. Cefoxitin and α-methyldopa absorption demonstrated a similar rank order in intestinal rings and the *in vivo* study, while cefoxitin absorption exceeded α-methyldopa absorption in mucosal cells.

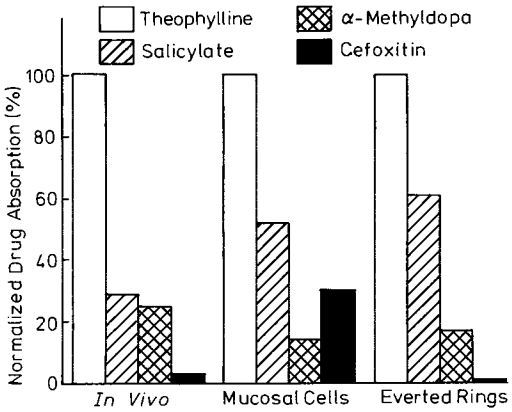

Fig. 2 Normalized theophylline, salicylate, α-methyldopa and cefoxitin absorption in mucosal cells, everted intestinal rings, and in *in vivo* studies. The data employed for calculating normalized absorption in mucosal cells and everted rings was obtained under identical experimental conditions (2 min incubation, 37°C, 5 mg/ml). Theophylline absorption was set at 100 per cent, and the absorption levels for the other drugs was normalized relative to theophylline.

Discussion

The use of *in vitro* models to examine drug absorption has been employed by a number of researchers (1–9). We have previously demonstrated that brush border membrane vesicles when compared to isolated cells and intestinal segments appear to have limited utility, primarily because of high variability in drug uptake (10). In this report, mucosal cells and intestinal rings have been further tested, and compared with *in vivo* drug absorption to determine the potential utility of these models as predictors of intestinal drug absorption.

Salicylate absorption is generally believed to occur via a passive diffusional mechanism (17, 18). Some evidence, however, has also suggested the possible involvement of an active transport component in salicylate absorption (19, 20). The results reported here tend to confirm and support a passive mechanism for salicylate absorption, in that drug uptake was not temperature-dependent and salicylate accumulation was non-saturable in the concentration range examined.

Schrogie et al. (21) have shown that cefoxitin, a semi-synthetic cephamycin antibiotic, is poorly available in several species (< 2 % in rats and mice, < 10 % in monkeys). Cefoxitin was absorbed to a limited extent in both cells and rings, although cellular cefoxitin uptake was erratic (e. g. decreased uptake with increasing incubation time and apparent increased absorption at lower temperatures). The decrease in cefoxitin absorption with increasing incubation times may be due to the length of time required to separate the cells from the incuba-

tion solution with the centrifugation technique. If cefoxitin absorption were very rapid, maximum levels may be obtained at the earliest time point examined (8.1 μg/mg protein at t = 0 min). The apparent high levels of cefoxitin in cells at t = 0 min may also reflect non-specific adsorption to the cellular surface that is not reversed during centrifugation through the sucrose-bovine serum albumin layer. Cefoxitin accumulation in rings was non-saturable in the concentration range tested, and not temperature-dependent, indicating that cefoxitin may be absorbed to a limited extent by passive absorption. The low uptake in these *in vitro* experiments, and the poor *in vivo* absorption, probably reflect the charged nature of the molecule (pKa 2.2) at physiological pH.

α-Methyldopa absorption in cells and rings was relatively low compared to salicylate or theophylline uptake, but did demonstrate some interesting characteristics. In the intestinal ring system, α-methyldopa absorption appeared to be saturable above 2 mg/ml (9.5 mM). Absorption was strongly temperature-dependent with both isolated cells and intestinal rings. The exact mechanism by which α-methyldopa is absorbed *in vivo* is unclear, but some evidence suggests the possible utilization of an amino acid transport mechanism in the gut wall (22–24). As such, a saturable and temperature dependent uptake process might be expected, and may be reflected in the intestinal ring studies reported here. This is currently being examined in greater detail.

Theophylline is known to be well absorbed *in vivo* (25). Similar observations have been made in this study with mucosal cells and rings. The absorption process would appear to be passive, since potential competitive compounds (xanthine, inosine) and metabolic inhibitors (2,4-dinitrophenol, ouabain) did not significantly reduce theophylline uptake in rings. Several workers have reported similar results (26, 27). Theophylline accumulation was also unaffected when the incubations were performed in sodium-free medium. Even though the data do not support an active transport mechanism, the temperature-dependence observed with theophylline uptake into rings remains enigmatic. It is unlikely that a general change in membrane permeability at reduced temperature is involved, since a similar temperature-dependence in ring transport studies was not observed with salicylate. The possible existence of a high affinity, low capacity active transport process for theophylline uptake cannot be precluded, however, especially in view of previously reported studies demonstrating active accumulation of pyrimidine and purines (28, 29). A high capacity passive absorption process could obscure a low capacity active transport system.

The data presented on temperature effects on salicylate, cefoxitin, α-methyldopa and theophylline accumulation must be interpreted with caution. Characteristically, a highly significant temperature-dependence is often interpreted as an indication of an energy-dependent process. It must be noted that temperature-dependence, without other supporting metabolic data or inhibitor studies, does not prove a process involves an active, energy-dependent component. This is apparent in the theophylline studies where metabolic inhibitors and potential competitive transport inhibitors did not significantly reduce theophylline accumulation in intestinal rings, even though a significant temperature-dependence was observed.

A comparison of drug absorption between the *in vitro* systems (isolated cells and intestinal rings) and the *in vivo* studies (Figure 2) demonstrated a good correlation in rank order of drug absorption between the *in vivo* results and the intestinal ring absorption data. The relative order of drug absorption in mucosal cells did not reflect *in vivo* absorption as accurately as the intestinal ring model. Considering the varying absorption characteristics of the drugs studied, the data suggest that the intestinal ring system may be an accurate indicator of the level of *in vivo* drug absorption.

In summary, although both mucosal cells and intestinal rings were shown to be capable of absorbing a variety of drugs, the use of intestinal rings appear to offer several advantages. Rings can be prepared with relatively simple procedures and are viable under incubation conditions for 10–20 min (10). Drug uptake studies can be performed at very short time intervals, and the variability is normally less than that observed with isolated cells. Unlike isolated cells, the serosal membrane of the mucosal cell in the ring preparation is not as readily accessible to drugs in the incubation solution. Finally, and perhaps most important, the data on drug absorption obtained with rings agree very closely with *in vivo* data. As such, rings may serve as a good screening model for intestinal drug absorption and as a mechanistic model to examine the basic controlling features of drug absorption at the mucosal barrier.

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Role of Peripheral Adrenergic Responsiveness in the Development of DOCA/NaCl Hypertension in Rats

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Abstract: Alterations in α- and β-adrenergic responsiveness were investigated prior to and during the development of hypertension in rats treated with desoxycorticosterone acetate and NaCl (DOCA/NaCl). The DOCA/NaCl rats became noticeably hypertensive (> 150 mm Hg) six weeks after the initiation of treatment. Prior to the development of hypertension, a reduced *in vivo* and *in vitro* β- and an enhanced α-adrenergic responsiveness of the DOCA/NaCl group resulted. At 2 and 12 weeks of the study, the dipsogenic response to isoproterenol was significantly attenuated in the DOCA/NaCl rats, whereas no difference in the dipsogenic response to 24 hour water deprivation was observed between control and DOCA/NaCl rats. Isoproterenol-induced relaxation of aortic smooth muscle from the DOCA/NaCl treated rats was significantly reduced at 4 weeks and further attenuated at 12 weeks of the study. However, aortic smooth muscle sensitivity to norepinephrine stimulation was significantly increased at 4 and 12 weeks of the study. These results suggest that alterations in both *in vivo* and *in vitro* α- and β-adrenergic responsiveness occur prior to establishment of hypertension of the DOCA/NaCl rats and that these alterations may have a role in the early stages of the development of this form of hypertension.

Elevation of blood pressure in both intact and uninephrectomized rats accompanies chronic administration of the mineralocorticoid hormone desoxycorticosterone (DOC) (1, 2). Suggested mechanisms responsible for DOC-induced hypertension include: an enhanced vascular reactivity (1, 3, 4); sodium retention and expansion of extracellular volume (5, 6); enhanced sympathetic nervous activity (7); alterations in the concentrations of electrolytes in the cell wall (2) and increased concentrations of catecholamines in the blood (8, 7).

Regardless of the ultimate pathogenic mechanism, the presence of sodium and the participation of the sympathetic nervous system in the development and initiation of DOC-induced hypertension have been supported by several experimental findings (2–4, 7, 9). An increase in *in vitro* α-adrenergic responsiveness (3, 4) and a decrease in *in vivo* β-adrenergic responsiveness in DOC/NaCl treated rats (10) have been reported. Additionally, an increase in peripheral sympathetic outflow and plasma catecholamines; a reduction in the number of β-adrenergic receptors (11, 12); and an increase in the number of α-adrenergic receptors (13) have been reported in hypertensive rats.

It has been suggested that a primary pathogenic factor in the development of this form of hypertension could be enhanced vascular reactivity observed in the DOCA/NaCl rat, since the increased responsiveness to α-adrenergic agonists occurs prior to the development of hypertension (3). Sodium deficiency in the DOC-treated rat prevented the occurrence of the enhanced vascular reactivity to norepinephrine (10). This and other reports (14) support the importance of salt in the change in vascular responsiveness. Rats treated with DOC-tap water (15) as well as thyroidectomized DOC/NaCl-treated rats (16) exhibit enhanced vascular reactivity without any change in blood pressure.

The purpose of the present study was to investigate if the alteration in both α- and β-adrenergic responsiveness occurs prior to as well as during the hypertensive state in the DOC/NaCl rats using both *in vivo* and *in vitro* techniques.

Methods

Sixty male Sprague Dawley rats (Blue Spruce Farms) were housed in groups of 2, in hanging stainless steel cages, in a room illuminated from 0700 to 1900 h and maintained at 24 ± 1°C. Food (Purina laboratory chow) was provided *ad libitum*.

Thirty of the rats were lightly anesthetized with ether and subcutaneously implanted with pre-weighed pellet of desoxycorticosterone acetate (DOCA: Sigma Chemical Co.). These pellets were made with an F. J. Stokes tablet maker. A 1% NaCl solution was provided *ad libitum*. The remaining thirty rats were sham treated, and tap water was provided *ad libitum*. Indirect systolic blood pressures (SBP) were measured every other week by means of a Narco-Bio systems transducer and physiograph, using the standard tail cuff technique.

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