

Responses of the Skin Microcirculation to Acetylcholine in Patients with Essential Hypertension and in Normotensive Patients with Chronic Renal Failure

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Key Words

Acetylcholine · Chronic renal failure · Cutaneous blood flow · Endothelium · Essential hypertension · Laser Doppler flowmetry

Abstract

Aims: To assess the endothelial function of the skin microcirculation in chronic renal failure (CRF) independent of hypertension, we investigated the changes of the cutaneous blood flow induced by iontophoretic delivery of acetylcholine (ACh) and of sodium nitroprusside (SNP) in CRF patients free from arterial hypertension and in patients with essential hypertension. **Methods:** The study included 20 patients affected by CRF (mean creatinine clearance 12 ± 2 ml/min) without arterial hypertension (mean blood pressure 96 ± 1 mm Hg), 15 patients affected by essential hypertension (mean blood pressure 124 ± 1 mm Hg), and 20 normal controls. The changes of skin blood flow following iontophoretic delivery of ACh and of SNP were measured by laser Doppler flowmetry. **Results:** Following maximal ACh or SNP delivery, the change of blood flow from the baseline was similar both in normals (683 ± 92 vs. $684 \pm 87\%$) and in CRF patients (778 ± 108 vs. $803 \pm 124\%$), whereas in the hyperten-

sives the response to ACh was lower than to SNP (434 ± 48 vs. $702 \pm 98\%$, $p < 0.01$). Since the third ACh delivery dose, the skin blood flow increments were significantly lower in the hypertensive than in the CRF or in the normal control groups, whereas no difference was observed between uremics and controls. **Conclusions:** The endothelium-dependent hyperemia following ACh iontophoretic delivery is impaired in the skin microcirculation of essential hypertensive patients, but this is not the case in CRF patients with no history of arterial hypertension. This suggests that CRF per se, independent of arterial hypertension, is not associated with endothelial dysfunction of skin microcirculation.

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Introduction

Factors frequently found in renal disease patients, such as arterial hypertension, hyperlipidemia, diabetes, and hyperhomocysteinemia, can negatively affect endothelial cell functioning. Since endothelium dysfunction may be an early manifestation of the atherogenesis process, it could be implicated also in the high cardiovascular risk reported in uremic patients [1–3]. Evidence exists that the

endothelium-dependent dilation of the brachial artery is impaired in adult dialysis patients and in children with chronic renal failure (CRF) [4–6]. On the contrary, experimental data failed to demonstrate similar findings in mesenteric arteries of uremic rats [7]. Moreover, up to date, endothelium-dependent vasodilation of small arterial vessels has not been studied in CRF patients. Several reports have clearly demonstrated that essential hypertension is associated with endothelial dysfunction both in the muscle vascular bed and in the skin microcirculation of the forearm [8–11]. Since it is well known that CRF patients are usually hypertensive, the question arises as to whether uremia ‘per se’ or the frequently associated arterial hypertension can negatively affect the endothelial cell function in uremics. Hence, the arterial hypertension and its pharmacological treatment could represent important confounding factors for the study of endothelial dysfunction in these patients.

The recent use of iontophoresis coupled with laser Doppler flowmetry [12] allows to assess the real-time changes of the skin blood flow in a noninvasive manner [13–15]. In addition, since the drug is delivered locally in the skin, systemic effects that could interfere with the skin microcirculatory responses are avoided.

Hence, to assess the small-vessel endothelial function in CRF independent of hypertension, we investigated the changes of cutaneous blood flow induced by the iontophoretic delivery of an endothelium-dependent (acetylcholine; ACh) and of an endothelium-independent (sodium nitroprusside; SNP) vasodilator in patients with advanced CRF, but free from arterial hypertension, and in patients with essential hypertension.

Patients and Methods

Subjects

The study population included 20 CRF patients free from arterial hypertension (CRF group), 15 patients affected by essential hypertension (EHT) with normal renal function (EHT group), and 20 normal control subjects (NC group). The three groups did not differ as far as the age was concerned (table 1).

Patients and subjects older than 65 years, heavy smokers (more than 5 cigarettes daily), or those affected by diabetes mellitus (fasting glucose levels >126 mg/dl) or with abnormally elevated cholesterol plasma levels (>240 mg/dl) were excluded. Subjects smoking up to 5 cigarettes daily were requested to refrain from smoking for 12 h at least before examination.

Blood urea nitrogen, creatinine, electrolyte, cholesterol, triglyceride, fasting glucose, total protein and albumin circulating levels, as well as hematocrit and arterial blood pressure values of the three groups are shown in table 1.

Table 1. Characteristics of the patient groups and controls (mean \pm SEM)

	Group		
	CRF	EHT	NC
Age, years	45 \pm 3	49 \pm 2	44 \pm 3
Cholesterol, mg/dl	183 \pm 10	188 \pm 4	174 \pm 3
Triglycerides, mg/dl	177 \pm 18	96 \pm 14	87 \pm 6
Fasting glucose, mg/dl	91 \pm 2	92 \pm 1	90 \pm 1
Creatinine, mg/dl	6.5 \pm 0.5**	0.8 \pm 0.0	0.8 \pm 0.0
Blood urea nitrogen, mg/dl	49 \pm 5**	13 \pm 1	14 \pm 1
Calcium, mg/dl	8.9 \pm 0.1*	9.4 \pm 0.1	9.3 \pm 0.1
Phosphate, mg/dl	4.2 \pm 0.2*	3.7 \pm 0.1	3.8 \pm 0.1
Potassium, mEq/l	4.4 \pm 0.1*	4.0 \pm 0.1	3.8 \pm 0.1
Sodium, mEq/l	140 \pm 1	141 \pm 1	140 \pm 1
Hematocrit, %	31.5 \pm 1.2**	40 \pm 1	39 \pm 1
Total proteins, g/dl	7.0 \pm 0.2	6.9 \pm 0.1	7.1 \pm 0.1
Albumin, g/dl	4.0 \pm 0.1	4.0 \pm 0.1	4.1 \pm 0.1
Blood pressure, mm Hg			
Systolic	131 \pm 3	172 \pm 3 [∞]	134 \pm 4
Diastolic	78 \pm 1	100 \pm 2 [∞]	78 \pm 2
Mean	96 \pm 1	124 \pm 1 [∞]	97 \pm 2

* $p < 0.01$, ** $p < 0.001$ vs. EHT and vs. NC groups.

[∞] $p < 0.001$ vs. CRF and NC groups.

The CRF group included 9 males and 11 females affected by CRF (creatinine clearance 12 ± 2 ml/min), on conservative treatment. The patients were selected because they were normotensive in the absence of antihypertensive treatment and free from a history of hypertension.

Diastolic blood pressure >85 mm Hg and/or systolic blood pressure >150 mm Hg was regarded as exclusion criteria, as well as the presence of nephrotic syndrome or severe hyperhydration. Patients treated with angiotensin-converting enzyme-inhibitors or with angiotensin II receptor antagonists, as antiproteinuric agents, were also not included in the study.

The underlying renal disease was tubulointerstitial nephritis in 7 cases, chronic glomerulonephritis in 3, polycystic kidney disease in 3, congenital bilateral hypoplasia in 1, and unknown in 6 cases.

The CRF patients were on conservative treatment with a standard low-protein (0.6 g/kg/day) diet or with a very-low-protein (0.3 g/kg/day) diet supplemented with essential amino acids and ketoanalogues [16], according to the residual renal function. Three out of the 20 CRF patients were treated with low doses of erythropoietin (2,000 U twice weekly).

The EHT group included 5 males and 10 females affected by untreated newly diagnosed EHT, with normal renal function (creatinine plasma level <1.2 mg/dl). Nine males and 11 females with normal renal function, normotensive and without familial history of essential hypertension, formed the NC group. All the subjects gave their informed consent for the study.

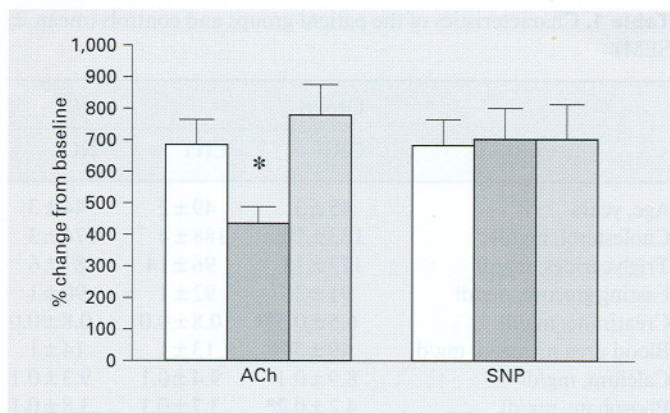


Fig. 1. Percentage change from baseline of the maximal skin blood flow following ACh or SNP iontophoretic delivery in the NC (open columns), EHT (hatched columns); and CRF (dotted columns) groups. * $p < 0.05$ vs. NC and CRF groups.

Procedures

Endothelium-dependent or endothelium-independent hyperemia was determined by the combination of iontophoresis and laser Doppler flowmetry.

Skin erythrocyte flow measurements were carried out in a temperature-controlled room ($22 \pm 1^\circ\text{C}$), with the subjects lying in supine position, after a 20-min acclimatization period. Before starting, the skin of the forearm was carefully cleaned using alcohol.

Iontophoresis has been used to deliver vasoactive substances across the skin of the forearm, avoiding the systemic effects that could interfere with the skin microcirculatory responses. In fact, this method allows to locally transfer charged drugs across the skin through a small electric current [17]. The current strengths chosen for our study were well tolerated by the majority of the subjects, whereas a mild prickling sensation was reported in a few cases. We have previously evaluated the effect of iontophoresis current itself on skin blood flow: negligible changes were induced by the same duration and strength of the electric current we used in the present study. A drug delivery electrode (PF 383; Perimed, Järfälla, Sweden) and an indifferent electrode (PF 384; Perimed) were used. The electrode chamber, with the laser probe placed in the middle, was attached to the volar surface of the forearm by a double-sided adhesive disk, at a 10-mm distance from the drug delivery electrode.

Before to be attached on the skin, the drug delivery electrode chamber was filled with $62.5 \mu\text{l}$ of 1% ACh solution and with $62.5 \mu\text{l}$ of 1% SNP solution. A battery-powered iontophoresis controller (Perilout 328, Power Supply) was used to provide the current for drug administration. After registration of the baseline flow, ACh was delivered using an anodal current: six doses (0.1 mA for 20 s each) followed by another two doses (0.2 mA for 20 s and 0.3 mA for 20 s) were delivered with a 60-second interval between each single dose. The 60-second interval was needed to achieve the plateau of the hyperemia induced by each ACh delivery.

After a 1-min recovery period, SNP was delivered using a cathodal current: two doses (0.1 mA for 20 s each) followed by one dose (0.2 mA for 20 s) with a 180-second interval between two successive doses. The 180-second interval was needed to achieve the steady state of the blood flow response following each SNP dose.

Before and during ACh or SNP delivery, the skin erythrocyte flow was measured by means of a laser Doppler flowmeter (Periflux PF4001, standard probe PF408, Perimed). The laser Doppler probe was fixed within the drug chamber to explore the same small area of the skin. A laser beam penetrates the skin and it is partially backscattered by moving blood cells. According to the Doppler principle, a frequency shift occurs, generating a signal that is linearly related to red cell flow, as predicted by theoretical and experimental models [18–20]. The laser Doppler outputs were recorded continuously by an interfaced computer equipped with acquisition software (PC Notebook; Zenith Data Systems, Taipei, Taiwan) and given as arbitrary units (perfusion units, PU). Calibration was performed by a device composed of colloidal latex particles, the Brownian motion of which provides the standard value. The skin blood flow was determined as the mean value recorded during 60 s at each delivery step. The absolute maximal response was defined as the flow reached after the last drug delivery.

The reproducibility of laser Doppler flowmetry has been studied in stable emulsion with an intra-assay coefficient of variation of about 6% [20]. In humans, the technique shows a coefficient of variation of 20–21% [21].

In order to eliminate baseline variability, the blood flow responses to ACh and SNP deliveries were expressed as percent change from the baseline [12, 22]. Similarly, the flow increment following each one of the eight iontophoretically applied ACh doses was expressed as percentage of the maximal endothelium-independent response to SNP as follows: $100 \cdot \text{ACh-induced flow} / \text{max SNP-induced flow}$.

Statistics

All results are expressed as mean \pm SEM. Statistical evaluation was performed using one-way Anova and the Student t test for paired data. The hyperemic responses following the eight ACh delivery steps were analyzed by Anova for repeated measures; Scheffé's test was applied for multiple-comparison testing. Differences were considered as statistically significant when $p < 0.05$.

Results

Figure 1 shows that the EHT patients have a blunted response to ACh, whereas no difference between groups has been observed as far as the responses to SNP were concerned. To confirm, in the CRF group, the absolute increment from baseline to maximal flow following ACh delivery was similar to that obtained by SNP delivery (30.8 ± 3.0 vs. 32.6 ± 4.2 PU, respectively; n.s.), as it occurred in the NC group (25.7 ± 5.6 vs. 25.9 ± 5.4 PU, n.s.). On the other hand, in the EHT group, the maximal ACh-induced increment of blood flow was significantly lower than that obtained by SNP (20.7 ± 2.3 vs. 39.1 ± 6.3 PU; $p < 0.01$). Although CRF patients had lower hematocrit values (table 1), they showed skin blood flow values similar to normals (5.1 ± 0.6 vs. 4.5 ± 0.6 PU; n.s.) at baseline.

Fig. 2. Skin blood flow changes following the eight doses of ACh iontophoretic delivery in the NC (solid line), EHT (dashed line) and CRF (dotted line) groups. The values are given as percentage of the maximal flow obtained by SNP delivery. * $p < 0.001$ vs. NC and vs. CRF groups.

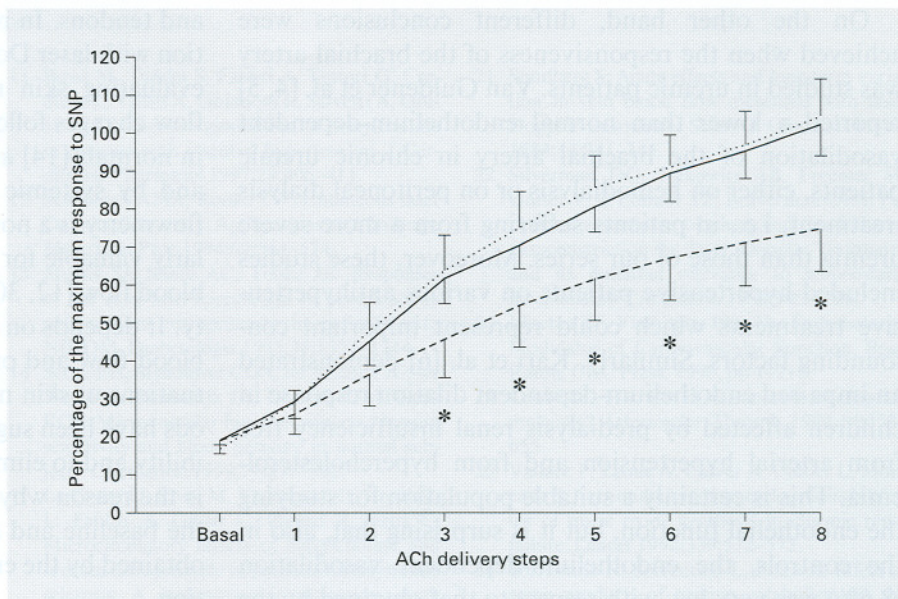


Figure 2 shows the cutaneous blood flow changes provoked by the eight doses of ACh delivery. The iontophoretic delivery of ACh was followed by a progressive increment of cutaneous blood flow in the three studied groups. However, in the EHT group the blood flow changes were significantly lower than in the NC or in the CRF groups, whereas no difference was found between the CRF patients and the NC subjects (fig. 2). In the latter two groups, the blood flow curves in response to ACh finally reached nearly 100% of the maximal endothelium-independent response provoked by the SNP delivery. In contrast, ACh delivery was able to induce approximately 75% of the maximal dilation in the EHT group.

Discussion

Our results indicate that essential hypertension, but not chronic renal failure, is associated with impaired endothelium-dependent vasodilation of the skin microcirculation.

Endothelial cells play a primary role in the local modulation of vascular tone by releasing vasodilating substances such as nitric oxide (NO) and dilator prostanoids, and contracting ones such as constrictor prostanoids and endothelins [23]. The study of the endothelium-dependent vasodilation in chronic uremia is interesting because endothelial dysfunction is linked with the initiation and acceleration of the atherosclerosis process [24], that is a

very important determinant of the progressing renal failure and of the morbidity and mortality rates of uremic patients. In chronic uremia, the endothelial function could be impaired by the increased serum concentrations of dimethylarginine, an endogenous inhibitor of NO synthetase [25], or of end products from protein catabolism able to inhibit NO release [26]. On the other hand, studies have demonstrated that NO production is enhanced in chronic uremia and that uremic serum is a potent agonist of NO release in cultured endothelial cells [27]. However, in the skin circulation, the vasodilator response following ACh iontophoretic delivery seems to be mainly mediated by endothelium-derived prostanoids rather than NO production [28, 29].

Moreover, chronic uremia is very frequently associated with arterial hypertension that, by itself, can account for endothelial damage. This prompted us to select a relatively rare population of adult uremic patients free from arterial hypertension, aiming at evaluating the endothelial function in chronic renal failure independent of hypertension.

The present study provides evidence that the endothelium-dependent hyperemic response to ACh is not impaired in the skin of chronic renal failure patients free from a history of arterial hypertension.

This is in agreement with results of the recent study performed by Thuraisingham and Raine [7] who demonstrated a preserved endothelium-dependent vasodilation in the mesenteric vessels of uremic rats.

On the other hand, different conclusions were achieved when the responsiveness of the brachial artery was studied in uremic patients. Van Guldener et al. [4, 5] reported a lower than normal endothelium-dependent vasodilation of the brachial artery in chronic uremic patients, either on hemodialysis or on peritoneal dialysis treatment, i.e., in patients suffering from a more severe uremia than those of our series. Moreover, these studies included hypertensive patients on various antihypertensive treatments which could represent important confounding factors. Similarly, Kari et al. [6] demonstrated an impaired endothelium-dependent dilation response in children affected by predialysis renal insufficiency free from arterial hypertension and from hypercholesterolemia. This is certainly a suitable population for studying the endothelial function, but it is surprising that, also in the controls, the endothelium-dependent vasodilation (8.6%) was very low with respect to that obtained by the direct stimulus (23.3%), suggesting a different power between the endothelium-dependent and the endothelium-independent stimuli used to induce brachial artery dilation.

In addition, the difference between brachial artery and cutaneous vessels can reflect also different mechanisms of endothelium-dependent vasodilation [28, 29]. A preserved endothelium-derived dilator prostanoid production in the skin vasculature of CRF patients, independent of NO generation changes, could contribute to explain our findings.

Although the CRF group showed lower hematocrit levels than normal, similar blood flow values at baseline were detected. This seems to exclude significant vasoconstriction or vasodilation that could overestimate or underestimate the responses to ACh or to SNP delivery in CRF patients.

Our results also show that patients with essential hypertension have a skin hyperemic response to iontophoretically applied ACh doses lower than that to SNP ones. This is in keeping with the impairment of endothelium-dependent dilation in the skin microcirculation, already described in hypertensive patients using an invasive method [11].

The present study represents the first attempt to evaluate the skin microcirculation responsiveness in patients with essential hypertension and in patients with CRF, but with no history of arterial hypertension, using the iontophoretic delivery of ACh and SNP. Iontophoresis is a technique commonly used to allow the local absorption of drugs across the skin tissue. In clinical practice it is employed to treat inflammatory diseases of skin, muscles,

and tendons. In recent years, iontophoresis, in combination with laser Doppler flowmetry, has been used also for evaluating skin microcirculation and cutaneous blood flow changes following different pharmacological stimuli in normals [14] and in patients affected by diabetes [13] and by systemic sclerosis [15]. Actually laser Doppler flowmetry is a noninvasive inexpensive method, particularly valuable for assessing the real-time changes in skin blood flow [12, 30], although it has a quite high variability. It depends on the many variables affecting cutaneous blood flow and on the intrinsic rapid and frequent fluctuations of skin microcirculation. Data processing methods have been suggested to increase day-to-day reproducibility and to eliminate baseline variability [12, 22]. This is the reason why the data are expressed as change from the baseline and as percentage of the maximal response obtained by the endothelium-independent SNP vasodilation.

Recently, this method has been further validated in patients with essential hypertension by infusing ACh and SNP into the brachial artery [11]. The blunted endothelium-dependent vasodilation observed using plethysmography in the muscle vascular bed was detected also by laser Doppler in the cutaneous microcirculation. This suggests that the skin vascular bed is a good 'window' to evaluate endothelial function and validates the laser Doppler as a useful noninvasive tool for measurement of blood flow changes [12].

In summary, our results demonstrate a preserved hyperemic response to iontophoretic delivery of ACh in the skin microcirculation of CRF patients, whereas it is impaired in patients affected by EHT.

This suggests that CRF per se, independent of arterial hypertension, is not associated with endothelial dysfunction of the skin microcirculation.

References

- Gris JC, Branger B, Vecina F, Al Sabadani B, Fourcade J, Schved JF: Increased cardiovascular risk factors and features of endothelial activation and dysfunction in dialyzed uremic patients. *Kidney Int* 1994;46:807-813.
- London GM, Guérin AP, Marchais SJ, Pannier B, Safar ME, Day M, Métivier F: Cardiac and arterial interactions in end-stage renal disease. *Kidney Int* 1996;50:600-608.
- Luke RG: Chronic renal failure – a vasculopathic state. *N Engl J Med* 1998;339:841-843.
- Van Guldener C, Lambert J, Janssen MJFM, Donker AJM, Stehouwer CDA: Endothelium-dependent vasodilatation and distensibility of large arteries in chronic haemodialysis patients. *Nephrol Dial Transplant* 1997;12(suppl 2):14-18.
- Van Guldener C, Janssen MJFM, Lambert J, Steyn M, Donker AJM, Stehouwer CDA: Endothelium-dependent vasodilatation is impaired in peritoneal dialysis patients. *Nephrol Dial Transplant* 1998;13:1782-1786.
- Kari JA, Donald AE, Wallace DT, Bruckdorfer KR, Leone A, Mullen MJ, Bunce T, Dorado B, Deanfield JE, Rees L: Physiology and biochemistry of endothelial function in children with chronic renal failure. *Kidney Int* 1997;52:468-472.
- Thuraisingham RC, Raine AEG: Maintenance of normal agonist-induced endothelium-dependent relaxation in uraemic and hypertensive resistance vessels. *Nephrol Dial Transplant* 1999;14:70-75.
- Linder L, Kiowski W, Bühler FR, Lüscher TF: Indirect evidence for the release of endothelium-derived relaxing factor in the human forearm circulation in vivo: Blunted response in essential hypertension. *Circulation* 1990;81:1762-1767.
- Panza JA, Quyyumi AA, Brush JE Jr, Epstein SE: Abnormal endothelium dependent vascular relaxation in patients with essential hypertension. *N Engl J Med* 1990;323:22-27.
- Taddei S, Virdis A, Mattei P, Salvetti A: Vasodilation to acetylcholine in primary and secondary forms of human hypertension. *Hypertension* 1993;21:929-933.
- Rossi M, Taddei S, Fabbri A, Tintori G, Credidio L, Virdis A, Ghiadoni L, Salvetti A, Giusti C: Cutaneous vasodilation to acetylcholine in patients with essential hypertension. *J Cardiovasc Pharmacol* 1997;29:406-411.
- Schabauer AMA, Rooke TW: Cutaneous laser Doppler flowmetry: Applications and findings. *Mayo Clin Proc* 1994;69:564-574.
- Morris SJ, Shore AC, Tooke JE: Responses of the skin microcirculation to acetylcholine and sodium nitroprusside in patients with NIDDM. *Diabetologia* 1995;38:1337-1344.
- Sernè EH, Stehouwer CDA, ter Maaten JC, ter Wee PM, Rauwerda JA, Donker AJM, Gans ROB: Microvascular function relates to insulin sensitivity and blood pressure in normal subjects. *Circulation* 1999;99:896-902.
- La Civita U, Rossi M, Vagheggini G, Credidio L, Fabbri A, Giusti C, Pasero R, Ferri C: Microcirculatory effects of acetylcholine and sodium nitroprusside in patients with systemic sclerosis: A preliminary investigation. *Arch Rheum Dis* 1998;6:231-239.
- Giovannetti S: Low-protein diets for chronic renal failure; in Giovannetti S (ed): *Nutritional Treatment of Chronic Renal Failure*. Boston, Kluwer Academic Publishers, 1989, pp 179-190.
- Harris R: Iontophoresis; in Licht E (ed): *Physical Medicine Library*. Connecticut, Elizabeth Licht, 1967, vol 4, pp 156-178.
- Bonner R, Nossal R: Model of laser Doppler measurement of blood flow in tissue. *Appl Optics* 1981;20:2097-2107.
- Nilsson GE, Tenland T, Oeberg PA: Evaluation of laser Doppler flowmeter for measurements of tissue blood flow. *IEEE Trans Biomed Eng* 1980;27:597-604.
- Tenland T, Salerud KG, Nilsson KG, Oberg PA: Spatial and temporal variations in human skin blood flow. *Int J Microcirc Clin Exp* 1983;2:81-90.
- Sundberg S: Acute effects and long-term variation in skin blood flow measured with laser Doppler flowmetry. *Scand J Clin Lab Invest* 1984;44:341-345.
- Silverman DG, Jotkowitz AB, Freemer M, Gutter V, O'Connor TZ, Braverman OIM: Peripheral assessment of phenylephrine-induced vasoconstriction by laser Doppler flowmetry and its potential relevance to homeostatic mechanisms. *Circulation* 1994;90:23-26.
- Lüscher TF, Vanhoutte PM: *The Endothelium: Modulator of Cardiovascular Function*. Boca Raton, CRC Press, 1990, pp 1-215.
- Cooke JP, Tsao PS: Is NO an anti-atherogenic molecule? *Arterioscler Thromb* 1994;14:653-655.
- Vallace P, Leone A, Calver A, Collier L, Moncada S: Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet* 1992;339:572-575.
- Sorrentino R, Sorrentino L, Pinto A: Effect of some products of protein catabolism on the endothelium-dependent and -independent relaxation of rabbit thoracic aorta rings. *J Pharmacol Exp Ther* 1993;266:626-633.
- Noris M, Benigni S, Boccardo P, Aiello S, Gaspari F, Todeschini M, Figliuzzi M, Remuzzi G: Enhanced nitric oxide synthesis in uremia: Implications for platelet dysfunction and dialysis hypotension. *Kidney Int* 1993;44:445-450.
- Noon JP, Walker BR, Hand MF, Webb DJ: Studies with iontophoretic administration of drugs to human dermal vessels in vivo: Cholinergic vasodilation is mediated by dilator prostanoids rather than nitric oxide. *Br J Clin Pharmacol* 1998;45:545-550.
- Khan F, Davidson NC, Littleford RC, Litchfield SJ, Struthers AD, Belch JJJ: Cutaneous vascular responses to acetylcholine are mediated by a prostanoid-dependent mechanism in man. *Vasc Med* 1997;2:82-86.
- Rossi M, Cupisti A, Morelli E, Tintori G, Fabbri A, Battini S, Vagheggini G, Barsotti G: Laser Doppler flowmeter assessment of skin microcirculation in uremic patients on hemodialysis treatment. *Nephron* 1996;73:544-548.