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Neutral endopeptidase inhibition, a new approach in the exploration of diabetic vasculopathy in rats

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Abstract

Diabetic complications are mostly vascular and involve alteration in blood vessel reactivity and permeability. The contribution of the latter dysfunction to the development of target organ damage has not been thoroughly examined. In this study, we verify the acute effect of three peptidase inhibitors (phosphoramidon: *N*-(α-rhamnopyranosylhydroxyphosphinyl)-Leu-Trp, thiorphan: 3-mercapto-2-benzyl-propanoylglycine, and SQ 28,603: *N*-(2-(mercaptomethyl)-1-oxo-3-phenylpropyl]-≤ *b*-alanine; each at a dose of 2 mg/kg), as well as captopril ([2*S*]-1-[3-mercapto-2-methyl-propionyl]-L-proline; 10 mg/kg), and an aminopeptidase inhibitor (amastatin: ([(2*S*,3*R*)-3-amino-2-hydroxy-5-methylhexanoyl]-Val-Val-Asp; 2 mg/kg) on capillary extravasation abnormalities in the streptozotocin-induced diabetic rat using the Evans blue method. Untreated diabetic rats exhibited a significant enhancement of Evans blue extravasation in the duodenum, upper bronchus, pancreas and skin (175 ± 19, 94 ± 4, 95 ± 9 and 47 ± 10 μg/g dry tissue respectively compared to 67 ± 9, 44 ± 5, 47 ± 4, and 6 ± 2 μg/g dry tissue). The three endopeptidase inhibitors normalized capillary permeability in all tissues. Also, treatment with captopril was associated with complete correction of capillary dysfunction in the skin and partially in the duodenum, upper bronchus, and pancreas. These findings indicate for the first time that the use of neutral endopeptidase inhibitors may be beneficial in preventing capillary abnormalities in this diabetic rat model.

Keywords: Diabetic vasculopathy; Capillary permeability; Neutral endopeptidase; Streptozotocin

1. Introduction

Complications of diabetes mellitus are essentially of vascular origin. Indeed, diabetic patients exhibit increased morbidity and mortality due to arterial hypertension, retinal microangiopathy, accelerated atherosclerosis, as well as coronary and renal failure (Christlieb, 1973; Jensen et al., 1987; Lorenzi and Cagliero, 1991; Porta et al., 1987). The endothelial cell plays an important role not only in controlling the passage of macromolecules across the intima of large vessels, potentially leading to the deposition of macromolecular material including lipoproteins (Ross, 1986), but also in modulating the transfer of albumin, fluid and small solutes from the vascular to the interstitial fluid volume in selective capillary networks (Sirois et al., 1990a,b; Lehoux et al., 1992), which may produce local tissue edema, alteration in the traffic of substrates and waste products between the vascular and cellular volumes, thereby potentially contribute to target organ damage (Aukland and Nicolaysen, 1981; Aukland and Reed, 1993).

Neutral endopeptidase (enkephalinase, EC 3.4.24.11) is a cell membrane-associated zinc metalloprotease which cleaves several peptides on the amino side of hydrophobic amino acids (Gafford et al., 1983; Skidgel et al., 1984). It is particulary abundant in membranes of the brush border epithelial cells of the intestine (Danielsen et al., 1980) and kidney (Benuck et al., 1981; Kerr and Kenny, 1974; Fulcher and Kenny, 1983), the lymph nodes and placenta; it is found also in significant concentrations in the lungs (Johnson et al., 1985; Llorens and Schwartz, 1981), testis, prostate, fibroblasts, neutrophils, articular cartilage chondrocytes, exocrine glands and various epithelial and endocrine cells (Roques et al., 1993). It is also localized

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on the surface of vascular endothelial cells (Llorens-Cortes et al., 1992). Like angiotensin I converting enzyme, neutral endopeptidase is concentrated on membranes of epithelial cells in a variety of tissues (Erdos, 1984). Angiotensin converting enzyme is located along the luminal surface of the vascular endothelium (Caldwell et al., 1976; Takada et al., 1982; Dubner and Ruda, 1992), where it can metabolize vasoactive peptides in the bloodstream, whereas neutral endopeptidase is found in the interstitial matrix (Johnson et al., 1985), where it could cleave circulating peptides crossing the endothelial barrier. Therefore, neutral endopeptidase could play a regulatory role in controlling inflammatory processes triggered by substances such as bradykinin, angiotensin II and substance P, which are released by injured tissue (Dubner and Ruda, 1992), and could be therefore potentially involved in the diabetic vasculopathy.

Two drugs have largely been used as neutral endopeptidase inhibitors: phosphoramidon, which is also an inhibitor of the endothelin converting enzyme (Télémaque et al., 1992), and thiorphan, which has no effect on the latter enzyme (Shimada et al., 1994). In order to increase the selectivity for neutral endopeptidase inhibition, various structural modifications of thiorphan were made and several thiorphan-derivatives have been investigated (Roques and Beaumont, 1990; Erdos and Skidgel, 1989). Another potent and selective neutral endopeptidase inhibitor is SQ 28,603: *N*-(2-(mercaptomethyl)-1-oxo-3-phenylpropyl]-≤ *b*-alanine, which has been recently reported to be without effect on the endothelin converting enzyme (Abassi et al., 1993). SQ 28,603 reduces blood pressure and produces natriuresis in experimental models of hypertension and heart failure (Seymour et al., 1991; Abassi et al., 1992).

Treatment with thiorphan has been reported to increase the reponse to ANP (atrial natriuretic peptide, Trapani et al., 1989). This peptide is structurally related to brain natriuretic peptide BNP (brain natriuretic peptide, Arimura et al., 1991; Ueda et al., 1991) and to CNP (C type natriuretic peptide), a newly identified peptide. This latter peptide is localized in the central nervous system (Komatsu et al., 1991a; Sudoh et al., 1990) as well as in the kidney, lung, and intestines of rats and humans (Komatsu et al., 1991b). CNP also has a strong hypotensive action, decreasing cardiac filling pressure and cardiac output (Stingo et al., 1992). CNP has a marked vasoactive effect in vivo and is associated with a guanylate cyclase receptor, preferentially on veins (Wei et al., 1993). This selective venodilator effect, if present in the microcirculation, might decrease intracapillary hydrostatic pressure, therefore decreasing albumin extravasation.

The aim of this study was to test the effect of three endopeptidase inhibitors (phosphoramidon, thiorphan and SQ 28603) on capillary permeability abnormalities in selected tissues of diabetic animals examined 4 weeks after injection of streptozotocin.

2. Materials and methods

Unanesthetized female Sprague-Dawley rats, weighing between 180 and 200 g, were used in this study: one control group and six diabetic groups ($n = 5–8$, in each group) injected with streptozotocin (65 mg/kg intraperitoneal). The onset of diabetes was confirmed by measuring blood glucose 3 days after streptozotocin. All animals injected with streptozotocin had blood glucose values higher than 20 mM. One diabetic group did not receive any treatment. The remaining 5 groups were injected on the day of the capillary permeability study with either intravenous phosphoramidon (2 mg/kg), thiorphan (0.4 and 2 mg/kg), SQ 28,603 (2 mg/kg), captopril (2, 10, and 50 mg/kg) or amastatin (2 and 10 mg/kg). All drugs were administered 40 min before the animals were killed. A dose of 2 mg/kg of phosphoramidon has been reported to be effective in previous studies (Lehoux et al., 1992). Dose-response studies were done with thiorphan, captopril and amastatin, in order to determine the effective dose of each inhibitor. We felt as unnecessary to perform another dose-response study with SQ 28603, since this inhibitor is derived from thiorphan.

Capillary permeability to albumin was measured using the extravasation of Evans blue dye. This technique has been described previously (Jancar et al., 1988) and is used in our laboratory (Lehoux et al., 1992). Briefly, the rats were injected with Evans blue (20 mg/kg) in the caudal vein, 10 min before they were killed and exsanguinated. The thorax was cut and the lungs were perfused with 15 ml of Krebs solution (10 ml/min) via the pulmonary artery to remove intravascular dye. Specimens from intrathoracic, splanchnic and peripheral organs, each group being perfused independently, were obtained: upper bronchus, lung parenchyma, heart, pancreas, duodenum, kidney, skin, and skeletal muscle (left quadriceps). Half of each tissue sample was dried at 60°C for 24 h, and a dry/wet weight ratio was calculated. The other half was placed in a formamide solution (4 ml/g wet tissue for 24 h) for dye extraction. The concentration of Evans blue was determined by spectrophotometry at 620 nm (Titertek Multiscan, Flow Laboratory) 24 h later. The concentration of Evans blue in each tissue was expressed in μg/g of dry tissue to avoid underevaluation due to tissue edema. The complete and tight Evans blue binding of serum albumin, extracellular equilibration of the marker, as well as complete extraction of the dye by formamide were all recently validated in our own (Sirois et al., 1990a,b; Lehoux et al., 1992) and other laboratories (Rogers et al., 1989; Patterson et al., 1992). The remote

possibility of incomplete removal of vascular Evans blue by exsanguination of animals would not appear to affect the absolute content of the dye, which is assumed to be distributed in the interstitium of the selected organs, since the contribution of the vascular space of these organs represents less than 8% of the total tissue volume (Wiig et al., 1992).

In another set of experiments, the rats were anesthetized with pentobarbital (50 mg/kg i.p). The right femoral vein was cannulated for administration of drugs, and the femoral artery for measurement of blood pressure 10, 20, 30 and 40 min after drug injection ($n = 4$ per drug). In unanesthetized rats of the diabetic treated group, glycemia was measured in blood samples taken from the caudal vein, before and 30 min after drug injection ($n = 4$ per drug).

Data are reported as mean values ± 1 S.E.M. Significance was determined by analysis of variance, and P values ≤ 0.05 were considered as valid.

Thiorphan (3-mercapto-2-benzyl-propanoglycine), amastatin ([(2*S*,3*R*)-3-amino-2-hydroxy-5-methyl-hexanoyl]-Val-Val-Asp) and Evans blue were all obtained from Sigma (St. Louis, MO, USA), phosphoramidon: *N*-(α-rhamnopyranosylhydroxyphosphinyl)-Leu-Trp from Peninsula Laboratories (Belmont, MA, USA), SQ 28,603 and captopril: [2*S*]-1-[3-mercapto-2-methyl-propionyl]-L-proline from Bristol-Myers Squibb laboratories (Princeton, NJ, USA).

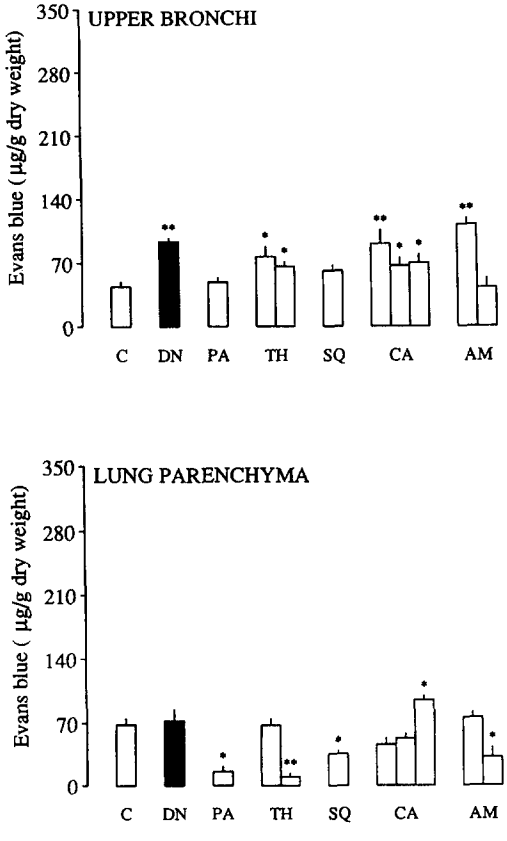


Fig. 1. Capillary permeability in the upper bronchi and lung parenchyma from control rats (C), untreated diabetic rats at 4 weeks (DN), diabetic rats treated with either phosphoramidon (PA; 2 mg/kg), thiorphan (TH; 0.4 and 2 mg/kg left and right bars respectively), SQ 28,603 (SQ; 2 mg/kg), captopril (CA; 2, 10, and 50 mg/kg left, middle, and right bars respectively), or amastatin (AM, 2, 10 mg/kg left and right bars respectively). Data represent the mean ± S.E.M of 5–6 experiments. * $P \leq 0.05$ and ** $P \leq 0.01$, statistically significant difference from control values.

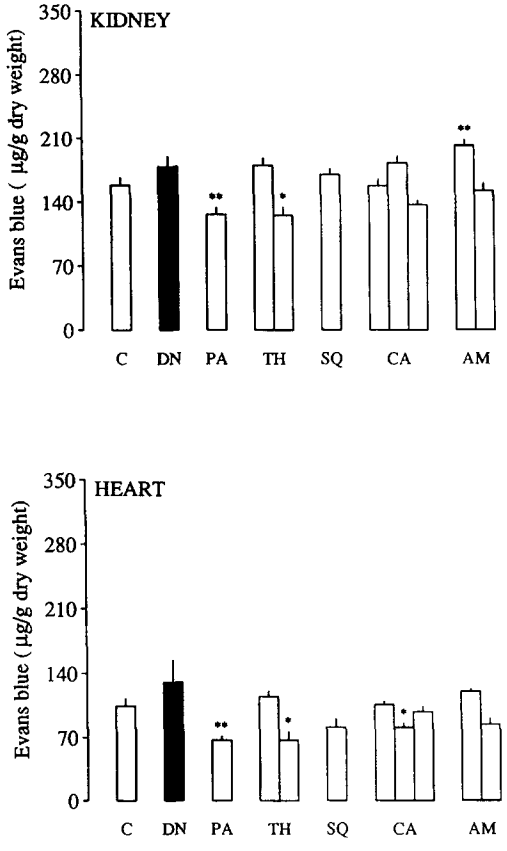
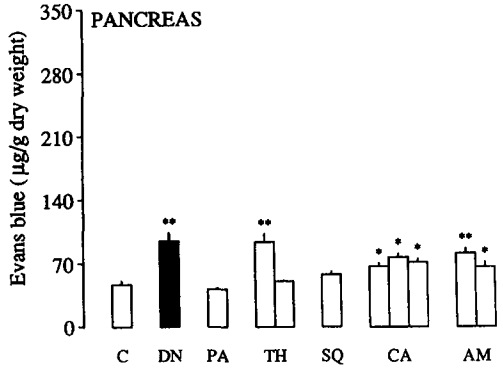
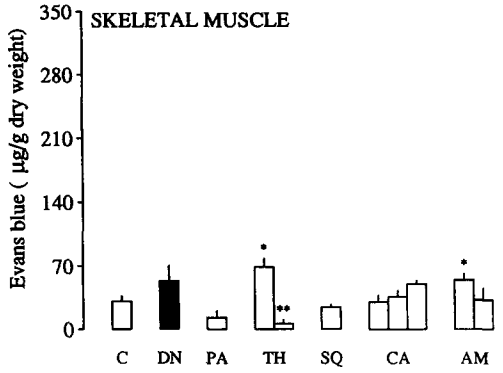


Fig. 2. Changes in vascular permeability in the kidney and heart from control rats (C), untreated diabetic rats at 4 weeks (DN), and treated diabetic rats as described for Fig. 1. Statistical significance is also as indicated for Fig. 1.

3. Results

As illustrated in Fig. 1, in upper bronchi, capillary permeability to Evans blue increased significantly from 44 ± 5 to 94 ± 4 µg/g dry tissue ($P \leq 0.01$). Treatment with the peptidase inhibitor phosphoramidon restored Evans blue extravasation back to baseline, 49 ± 5 µg/g dry tissue. Capillary permeability was normalized either completely with SQ 28,603 (61 ± 6 µg/g dry tissue) or partially with thiorphan (66 ± 5 µg/g dry tissue, $P \leq 0.05$). In the lung parenchyma, there was no significant increase in capillary permeability at 4 weeks. Treatment with phosphoramidon decreased permeability to under the control value (16 ± 5 vs. 72 ± 12 µg/g dry tissue, $P \leq 0.05$). Evans blue extravasation decreased from 72 ± 12 to 10 ± 4 µg/g dry tissue ($P \leq$





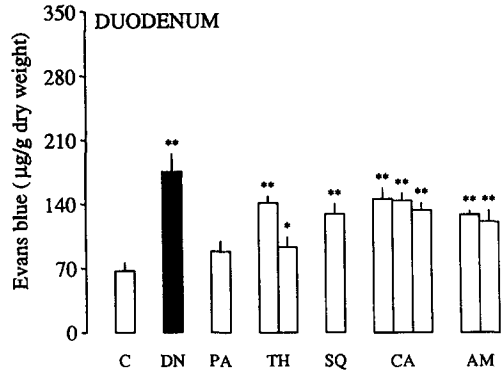


Fig. 3. Changes in vascular permeability in the pancreas and duodenum from control rats (C), untreated diabetic rats at 4 weeks (DN), and treated diabetic rats as described for Fig. 1. Statistical significance is also as indicated for Fig. 1.

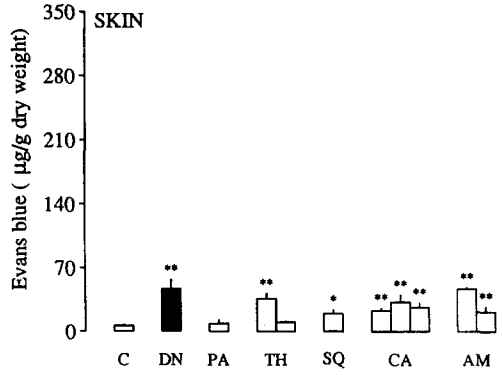


Fig. 4. Changes in vascular permeability in skeletal muscle and skin from control rats (C), untreated diabetic rats at 4 weeks (DN), and treated diabetic rats as described for Fig. 1. Statistical significance is also as indicated for Fig. 1.

0.01) with thiorphan and to 36 ± 3 μg/g dry tissue ($P \leq 0.01$) with SQ 28,603. Treatment with captopril (50 mg/kg) increased the Evans blue concentration to 95 ± 4 μg/g dry tissue ($P \leq 0.05$).

In the kidney and the heart, there was no significant change in permeability to albumin at this stage (Fig. 2). Treatment with phosphoramidon decreased albumin permeation from 178 ± 11 to 126 ± 7 μg/g dry tissue ($P \leq 0.01$) and from 130 ± 24 to 67 ± 4 μg/g dry tissue ($P \leq 0.01$) respectively. Administration of thiorphan led to a decrease in Evans blue leakage in the kidney and in the heart to 125 ± 9 μg/g dry tissue ($P \leq 0.05$) and to 67 ± 9 μg/g dry tissue ($P \leq 0.05$) respectively. SQ 28,603 did not induce any changes in permeability in the kidney or in the heart. Captopril had no effect in either tissue but caused a slight decrease to 81 ± 4 μg/g dry tissue in the heart ($P \leq 0.05$), at the dose of 10 mg/kg (Fig. 2).

In the pancreas and the duodenum, there was a significant increase in Evans blue permeability, from

Table 1
Effect of phosphoramidon (PA), thiorphan, SQ 28,603, amastatin and captopril on capillary permeability in kidney (KD), lung parenchyma (LP), heart (HT), duodenum (DU), upper bronchi (UB), pancreas (PC), skeletal muscle (SM) and skin (SK) in normal non-diabetic rats ($n = 6$ each experiment)

	Control	PA	Thiorphan	SQ28603	Amastatin	Captopril
KD	158 ± 8	165 ± 2	197 ± 6 [b]	187 ± 9 [a]	200 ± 9 [b]	159 ± 8
HT	104 ± 8	89 ± 7	128 ± 5	109 ± 6	111 ± 6	88 ± 5
LP	68 ± 7	46 ± 2 [a]	79 ± 8	27 ± 3 [b]	52 ± 3	53 ± 6
DU	67 ± 97	5 ± 8	97 ± 5 [a]	118 ± 9 [b]	92 ± 10 [a]	76 ± 3
PC	47 ± 4	57 ± 6	66 ± 3 [a]	46 ± 4	66 ± 4 [a]	62 ± 4 [a]
UB	44 ± 5	55 ± 9	78 ± 5 [a]	65 ± 3 [a]	56 ± 89	92 ± 5 [b]
SM	30 ± 6	58 ± 8 [a]	26 ± 5	10 ± 3 [b]	29 ± 9	19 ± 3
SK	6 ± 2	30 ± 8 [b]	23 ± 4 [b]	12 ± 3	24 ± 5 [b]	25 ± 2 [b]

Statistical significance: [a] $P \leq 0.05$ and [b] $P \leq 0.01$.

Table 2
Blood pressure (BP) and blood sugar (BS) measurements in control, diabetic untreated rats, and diabetic rats that received either phosphoramidon (PA), thiorphan, or SQ 28603 ($n = 4$ each experiment)

	Control	Diabetic non-treated	Diabetic + PA	Diabetic + thiorphan	Diabetic + SQ 28603
BP (mm Hg)	133/92 ± 2	108/85 ± 3 [b]	110/85 ± 3 [b]	115/90 ± 3 [b]	110/90 ± 2 [b]
BS (mM)	5–7	≥ 20	≥ 20	≥ 20	≥ 20

Significance as in Table 1.

47 ± 4 to 95 ± 8 μg/g dry tissue, and from 67 ± 9 to 176 ± 18 μg/g dry tissue respectively. Phosphoramidon corrected completely this increase. The dose of 2 mg/kg of thiorphan normalized completely this elevation of permeability in the pancreas and partially in the duodenum (88 ± 11 μg/g dry tissue, $P \leq 0.05$). Treatment with captopril failed to affect permeability in the two tissues (Fig. 3).

Capillary permeability was also evaluated in the skeletal muscle and the skin (Fig. 4). In skeletal muscle, capillary permeability to Evans blue remained normal at this stage of diabetes. Phosphoramidon did not induce any changes. Treatment with thiorphan at 0.4 mg/kg increased slightly the concentration of Evans blue in this tissue (69 ± 9 μg/g dry tissue, $P \leq 0.05$) and decreased it to 6 ± 4 μg/g dry tissue ($P \leq 0.01$) at the concentration of 2 mg/kg. Finally, treatment with either SQ 28,603 or captopril did not affect permeability in skeletal muscle. In the skin, there was an increase in permeability from 6 ± 2 to 47 ± 10 μg/g dry tissue ($P \leq 0.01$). Phosphoramidon as well as thiorphan restored completely Evans blue extravasation back to baseline values. The values averaged 13 ± 7 and 10 ± 1 μg/g dry tissue, respectively. Treatment with SQ 28,603 reduced capillary permeability in this tissue to 20 ± 4 μg/g dry tissue, $P \leq 0.05$. The angiotensin converting enzyme inhibitor failed to normalize permeability abnormalities in this tissue.

Another group of diabetic rats was treated with an aminopeptidase inhibitor to find out if the effects observed were specific to endopeptidases. The results reveal that administration of amastatin failed to normalize the increased permeability observed in the pancreas, the duodenum (Fig. 3) and the skin (Fig. 4). However, treatment with 10 mg/kg of amastatin decreased permeability in the upper bronchi to baseline value (42 ± 10 μg/g dry tissue) and in the lung parenchyma below baseline to 32 ± 11 μg/g dry tissue, $P \leq 0.05$ (Fig. 1).

To rule out an eventual intrinsic action of the inhibitors used in this study on baseline Evans blue leakage, we tested their effect in normal rats ($n = 6$ each). Phosphoramidon had no effect on Evans blue extravasation except for a small decrease in lung parenchyma from 68 ± 7 to 46 ± 2 μg/g dry tissue ($P \leq 0.05$) and a rise in the skin, from 6 ± 2 to 30 ± 8 μg/g dry tissue, $P \leq 0.01$ (Table 1). Thiorphan injected into normal rats increased Evans blue leakage in the upper bronchus, heart, pancreas, duodenum and skin. Values were 78 ± 5 ($P \leq 0.05$), 128 ± 5, 66 ± 3 ($P \leq 0.05$), 97 ± 5 ($P \leq 0.05$) and 23 ± 4 μg/g dry tissue, respectively ($P \leq 0.01$). This inhibitor did not induce any changes in the lung parenchyma, heart and skeletal muscle. Treatment of normal rats with SQ 28,603 decreased capillary permeability to albumin in the lung parenchyma (26 ± 3 μg/g dry tissue, $P \leq 0.05$) and skeletal muscle (10 ± 3 μg/g dry tissue, $P \leq 0.01$) and increased it in the upper bronchi to 65 ± 3 μg/g dry tissue ($P \leq 0.05$) and in the duodenum to 118 ± 9 μg/g dry tissue ($P \leq 0.05$) (Table 1). Captopril failed to produce any change in permeability in normal rats in most tissues examined, except for an increase in the upper bronchi (92 ± 5 μg/g dry tissue, $P \leq 0.05$) and in the skin (25 ± 2 μg/g dry tissue, $P \leq 0.05$). Finally, treatment of normal rats with amastatin enhanced capillary permeability in three tissues, the duodenum (92 ± 10 μg/g dry tissue, $P \leq 0.05$), pancreas (66 ± 4 μg/g dry tissue, $P \leq 0.05$) and skin (24 ± 5 μg/g dry tissue, $P \leq 0.01$).

In a separate series of experiments, we tested the effect of all drugs utilized on systemic blood pressure. A decrease in blood pressure was observed in diabetic rats compared to normal animals (108/85 ± 3 vs. 133/95 ± 2 mm Hg). All agents utilized were found not to affect peripheral blood pressure. Similary no effect of these treatments on blood sugar was documented. All diabetic treated rats remained hyperglycemic (Table 2).

4. Discussion

The consequences of increasing albumin extravasation in selected capillary networks, thereby potentially causing local tissue edema, can be examined from the point of view of interstitial fluid volume dysfunction. Any alteration in the physico-chemical characteristics of the interstitium (albumin exclusion space, proteoglycan composition), induced by abnormal capillary extravasation and/or removal of albumin by lymphatic drainage, is likely to affect the physiological function of this strategic fluid volume. Such an alteration may therefore become a threat to the cellular life of organs so affected (Aukland and Nicolaysen, 1981; Aukland

and Reed, 1993; Jackson et al., 1991). It is of interest that most tissues where increased Evans blue extravasation was documented in the present study are well-known target organs of diabetes mellitus in humans.

Neutral endopeptidase inhibitors were shown to be useful in several disorders of the vascular system including heart failure and arterial hypertension (Tamburini et al., 1989). Their effect seems to be related to a decrease in the breakdown of ANP, a potent vasodilator peptide. Kugiyama et al. (1993) showed that neutral endopeptidase inhibition restores endothelial dysfunction in a rabbit model of atherosclerosis, suggesting a role of neutral endopeptidase in atherogenesis, and proposed neutral endopeptidase inhibitors as potential therapeutic tools in the prevention of atherosclerosis. The results obtained in the present study reveal for the first time the beneficial effect of neutral endopeptidase inhibitors on endothelial cell dysfunction in an experimental model of diabetes mellitus. Indeed, treatment of diabetic rats with three different selective neutral endopeptidase inhibitors restored capillary permeability to baseline values in the upper bronchi, pancreas, duodenum, and skin, while treatment of normal rats with either phosphoramidon, thiorphan or SQ 28603 enhanced capillary permeability in the same tissues as well as in the kidney. The latter effect might be related, at least in part, to an increase in ANP and/or bradykinin level as these peptides have been shown to increase permeability in several models (Regoli et al., 1993; Valentin et al., 1993). No effect was observed in the heart of normal rats.

Thiorphan corrected capillary permeability abnormalities, as did phosphoramidon. These results suggest that endothelin is not the peptide primarily involved in this capillary permeability disorder. In normal rats, treatment with phosphoramidon or SQ 28603, but not with thiorphan, decreased capillary permeability in the lung parenchyma, a highly vascularized tissue. This effect could be related to inhibition of endothelin converting enzyme, which would decrease pulmonary endothelin-1 level and thus decrease permeability in this organ.

Treatment with captopril normalized capillary permeability, partially in the duodenum, upper bronchi, pancreas, and completely in the skin. This effect may be either due to inhibition of angiotensin II production, or due to kinin accumulation. In normal rats, captopril had no effect in the kidney, heart, lung parenchyma, duodenum, and skeletal muscle. In pancreas, upper bronchi and skin, there was a significant increase which could be related to the accumulation of kinins.

The fact that we observed a greater effect with thiorphan than with captopril suggests that the effect is related to inhibition of neutral endopeptidases rather than inhibition of angiotensin converting enzyme. Correction of capillary permeability abnormalities with SQ 28,603, a potent selective neutral endopeptidase inhibitor, confirms this hypothesis. The fact that the aminopeptidase inhibitor (amastatin) failed to normalize capillary permeability suggests that the correction of endothelial dysfunction obtained in the diabetic rat model results from specific inhibition of neutral endopeptidase.

Bucala et al. reported that nitric oxide (NO) is quenched by advanced glycosylation end-products, which are elevated in conditions of sustained hyperglycemia, such as diabetes mellitus (Bucala et al., 1991). NO depletion has been postulated to be implicated in the increased permeability observed in diabetes. NO production might be enhanced by the increased concentration of bradykinin in the presence of neutral endopeptidase inhibitors. Inhibition of endopeptidase action redirects the degradation of peptides to other peptidase pathways, such as those involving aminopeptidase and carboxypeptidase. It is possible that the arginine residue released from kinins by carboxypeptidase action could theoretically be used to produce NO by the NO synthase pathway, thereby normalizing capillary permeability, as observed in a previous study by Filep et al. (1993) in rat coronary microcirculation.

Macromolecule and fluid movement from the vascular compartment towards the interstitium is dependent on two major physiological determinants: (1) changes in endothelial cell permeability per se through dilatation or contraction of these cells, leading to formation of intercellular spaces, facilating fluid and solute movements across the capillary wall; (2) alteration in the capillary hydrostatic pressure, determined by the difference between pre-and post-capillary vascular resistances, which under a variety of vasoactive stimuli will lead to enhanced or reduced net forces for fluid and solute movements towards the interstitial space.

None of the peptides known to be inactivated by endopeptidases was associated with decreased capillary permeability. ANP is known to increase capillary permeability in vivo. The ectoenzyme neutral endopeptidase is localized on the endothelial cell surface and breaks down ANP. The endothelium may limit the action of CNP by degrading this peptide also. Receptors for ANP are expressed in endothelial cells, whereas receptors for CNP are expressed in vascular smooth muscle cells. This peptide has been shown to vasodilate veins rather than arteries (Wei et al., 1993) and, therefore, might play an important role in the control of vascular tone. By analogy with the effects documented in the macrocirculation, if the same pattern is present in the microcirculation, this peptide would diminish preferentially post-capillary resistance, leading to a decrease in capillary hydrostatic pressure and the movement of fluid and macromolecules towards the interstitial compartment. This hypothesis is consistent with

the results of in vivo studies by Stingo et al. (1992), who reported that CNP, like NO, mediates its effects via cGMP. Therefore, CNP may be anticipated to have actions on vascular permeability similar to those of NO.

In conclusion, this study demontrates for the first time the beneficial effect of neutral endopeptidase inhibitors on capillary permeability dysfunction in the streptozotocin-induced diabetic rat model. These findings provide a new approach to examine the pathophysiology of diabetic vasculopathy and may open up new ways of controlling the morbid consequences of altered capillary permeability in human diabetes mellitus. Further studies are required to elucidate whether NO and/or CNP are mediators of the phenomenon described above.

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