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INFLUENCE OF MUZOLIMINE ON ARTERIAL WALL ELASTIN

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Abstract—Muzolimine, 3-amino-1-(3,4-dichloro-α-methylbenzyl)-2-pyrazolin-5-one, an antihypertensive and diuretic drug, accumulates in the arterial tissue of rats and dogs after oral administration. Two weeks after the administration of 3 mg [^{14}C]muzolimine, the aorta of rats contained 60–300 times more ^{14}C-radioactivity/weight unit than the skin or tail tendon. The ^{14}C-radioactivity was exclusively bound to the isolated aortic elastin and corresponded to 0.04% of the applied muzolimine dose. Up to *ca* 250 ng bound muzolimine/mg elastin was found in the aorta of dogs treated with non-labelled muzolime for 52 weeks. The elastin-bound [^{14}C]muzolimine was not extractable by organic solvents or by weak acids or bases but was released in a soluble form by pancreatic elastase and extracted from the elastase digest by dichloromethane. In the dichloromethane extract muzolimine was detected by HPLC and HPTLC, and was identified by mass spectrometry. Muzolimine pretreatment of rats for 2 months did not influence the elastin content of arterial tissue or [^{3}H]glycine incorporation into aortic elastin under organ culture conditions, but after labelling the elastin with [4,5-^{3}H]lysine, the [^{3}H]desmosine and [^{3}H]-isodesmosine isolated from the elastin of muzolimine-pretreated rats and incorporated under organ culture conditions was lower than that of control animals. In addition, aortic elastin of rats pretreated for 2 months with 800 ppm muzolimine in the diet was more resistant to elastase degradation. This effect might give some implications for muzolimine in the therapy of cardiovascular disorders with impaired arterial elastin metabolism.

The pyrazolinone derivative muzolimine has been introduced as an effective diuretic drug [1–4]. In patients suffering from chronic renal failure and/or hypertension, muzolimine has been shown to have a saluretic activity similar to or better than that of furosemide [2, 4]. Whereas the saluretic action of muzolimine results from its influence on the thick ascending limb of Henle's loop, its antihypertensive effect is probably due to a peripheral vascular action [3, 5].

In addition, it has been shown that after [^{14}C]-muzolimine administration, radioactivity was bound to the connective tissue, predominantly in the vessel wall [6]. As it has been shown that cardiovascular disorders might be associated with an impaired connective tissue metabolism, at least in experimental hypertension [7, 8], the influence of muzolimine on connective tissue should be clarified. Hence, we addressed ourselves to the questions to which of the components of vascular connective tissue muzolimine is bound, whether unchanged muzolimine is detectable and, if so, whether muzolimine could interact which arterial tissue or influence its metabolism.

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MATERIALS AND METHODS

Muzolimine, 3-amino-1-(3,4-dichloro-α-methylbenzyl)pyrazolin-5-one, and muzolimine labelled with ^{14}C at C-4 of the pyrazoline (sp. radioact. 48.2 μCi, 1.78 MBq/mg) were supplied by Pharma Research Laboratories Bayer Ag (Wuppertal, F.R.G.). [2-^{3}H]Glycine (sp. radioact. 10–30 Ci/mmole, 0.37–1.1 TBq/mmole) and L-[4,5-^{3}H]lysine monohydrochloride (sp. radioact. 75–100 Ci/mmole, 2.7–4.7 TBq/mmole) were purchased from Amersham/Buchler (Braunschweig, F.R.G.); pancreatic elastase (porcine pancreatopeptidase E, EC 3.4.21.11, 120 U/mg) from Serva (Heidelberg, F.R.G.). Desmosine and isodesmosine were a gift from Dr. B. C. Starcher (St. Louis, MO). All other chemicals were of analytical grade or the best grade available and were purchased from Boehringer (Mannheim, F.R.G.), Merck (Darmstadt, F.R.G.), and Serva (Heidelberg, F.R.G.).

Male Sprague–Dawley rats (45 days old) were maintained on a standard diet (control) or on the same diet containing 800 ppm muzolimine for various time periods. At the time indicated, the animals were sacrificed and the aorta, skin from the abdominal area and the tail tendon were removed. In one series of experiments, each rat was given as a single dose 3 mg [^{14}C]muzolimine by oral administration 10 days prior to sacrifice.

One female beagle dog (10.5 kg) received a daily

oral dose of 3 mg/kg unlabelled muzolimine for 5 weeks followed by a single oral dose of 10 mg/kg [^{14}C]muzolimine of the labelled drug in a gelatine capsule. The animal was sacrificed 10 days thereafter and the aorta and the ligamentum nuchae were removed.

Unlabelled muzolimine at doses of 1, 3 and 10 mg/kg body wt was orally administered in gelatine capsules to beagle dogs for 52 weeks. At the end of the experiment, samples of the aorta were taken and stored at *ca* −20°.

Elastin was isolated according to Rasmussen *et al.* [9] with the following modifications: the aorta, skin and tail tendon were dehydrated and delipidated by extraction with chloroform–methanol 3:1 (v/v). Batches of 25 mg of the lipid-free dry weight of tissue were extracted with 5 ml of 8 M urea for 24 hr and the extract was monitored for radioactivity. After several washings, the tissue was suspended in 3 ml of 97% formic acid. Cyanogen bromide was added in excess (approximately 20 mg) and the mixture was kept at room temperature for 24 hr, then diluted with 2 vols. of distilled water, and filtered through a Whatman 3 MM filter pad of 2.5 cm diameter. The residue was exhaustively washed with water and lyophilized. The filtrate was monitored for radioactivity.

Elastin was determined from the residue gravimetrically. For elastase degradation the isolated elastin preparations were dried, ground and passed through a nylon sieve (100 μm meshes). The radioactivity of the cyanogen bromide degradation products and acid-hydrolysed elastin was determined by scintillation spectrometry (Packard Tri-Carb Model 3390/TT, Frankfurt, F.R.G.).

For organ culture, the freshly dissected aorta (50–75 mg) of control and muzolimine-pretreated rats was maintained in 10 ml Eagle's MEM medium supplemented with 10% fetal calf serum in the presence of 25 μCi [^{3}H]glycine or 25 μCi [4,5-^{3}H]lysine/ml medium for 5 days under conditions previously described [10]. No muzolimine was added to the culture medium.

Amino acid analysis was performed by routine methods (Amino Acid Analyser Beckman Multichrom B, München, F.R.G.). Desmosine and isodesmosine were isolated and assayed after acid hydrolysis of the elastin (6 M HCl, 110° under nitrogen for 24 hr) using the paper chromatographic method outlined by Starcher [11]. Enzymatic degradation of elastin or [^{14}C]elastin prepared from rats pretreated with [^{14}C]muzolimine was carried out by incubation in the presence of pancreatic elastase (2 U elastase/mg elastin) in Tris–HCl buffer, pH 8.0. The digest was centrifuged and submitted to HPLC. Aliquots of the digest were extracted with dichloromethane. The organic layer was evaporated under argon, the residue dissolved in methanol and injected into a high pressure liquid chromatograph (Hewlett Packard 1084 B, Waldbronn, F.R.G.) equipped with a RP 18 column from Merck (Darmstadt, F.R.G.). Muzolimine was eluted by a water–methanol gradient, starting with 20% methanol and 80% water and increasing the methanol content of the eluant to 60% within 15 min and further to 100% within 5 min. The eluant was monitored by a UV detector at λ = 266 nm. Fractions of the eluant (one fraction/min) were collected and used for radioactivity determination (liquid scintillation counter PW 4700, Philips, Eindhoven, The Netherlands) or for mass spectrometry. Mass spectra were taken on a mass spectrometer (Kratos M 30, Kratos Analytical Instruments, Manchester) with the following operating conditions: electron impact ionization, 70 eV; source temperature, 200°; direct inlet system, probe temperature 130°. A multiple-ion monitoring technique was chosen for detection. The two significant and most intense ions in the spectrum of muzolimine, *m/z* 173 ($Cl_2C_6H_3CHCH_3$) and *m/z* 99 ($C_3H_5N_3O$) were chosen for recording on an oscillograph. The resolution was *ca* 3000 (10% valley) to eliminate other ions of the same nominal mass but different elemental compositions.

Non-labelled muzolimine was determined by high performance thin-layer chromatography (HPTLC) as follows: 1.0 ml of the elastase-degraded elastin incubation mixture was extracted with 150 μl of dichloromethane, and 40 μl of the dichloromethane extract were spotted onto a precoated silica gel 60 HPTLC plate (10 × 20 cm). After developing in chloroform–ethanol, 9:1 (v/v), the plate was dipped for 5 sec into a solution of 4-dimethylaminocinnamaldehyde and heated for 5 min at 100°. The developed blue spots (R_f 0.41) were quantified at 610 nm at a densitometer (Zeiss KM III, Oberkochen, F.R.G.) connected to an integrator [12]. A calibration curve of muzolimine standards from the same thin-layer plate was used.

Comparative data were analysed by Student's *t*-test. A P value of less than 0.05 was considered significant.

RESULTS

[^{14}C]*Muzolimine activity in arterial tissue*

The elastin tissue concentration was determined in the aorta, skin, tail tendon and ligamentum nuchae. The elastin content of the aorta thoracica amounted to 44.6 ± 3.1% (rat) and 48.1 ± 4.2% (dog), that of the ligamentum nuchae (dog) to 78.5% of the dry weight of tissue. Rat skin contained 65% collagen and 3% elastin of the dry weight of tissue. In the tail tendon, the elastin content determined after delipidation, urea extraction and cyanogen bromide degradation was less than 1% of the dry weight.

One week after administration of 3 mg/kg body wt [^{14}C]muzolimine, the aorta of rats contained 60 and 300 times more ^{14}C-radioactivity than the skin and tail tendon. 97% of the ^{14}C-radioactivity incorporated into the arterial wall was associated with the elastin fraction of tissue (Table 1).

The elastin isolated from the aorta and from the ligamentum nuchae of a dog pretreated with non-labelled muzolimine for 5 weeks and 10 mg [^{14}C]-muzolimine thereafter had a specific radioactivity of 7200 and 4300 cpm/mg elastin and contained 96.6 and 93% of the radioactivity incorporated into these tissues (Table 1). The amino acid analysis identified the elastin fraction of the aortic tissue as pure elastin (Table 2). From the specific ^{14}C-radioactivity of the administered muzolimine and that of the isolated aortic ^{14}C-labelled elastin, it was calculated that the

Table 1. Incorporation of ^{14}C-radioactivity into tissue subfractions of the aorta, ligamentum nuchae, skin and tail tendon of rats and a beagle dog after 3 mg and 10 mg [^{14}C]muzolimine/kg body wt, respectively (sp. radioact. 48.2 μCi/mg)

Organ	^{14}C-Radioactivity (cpm per 100 mg dry wt)	Percentage of ^{14}C-radioactivity incorporated into				Sp. radioact. of elastin (cpm/mg)
		$CHCl_3$–MeOH	8 M urea extract	BrCN–soluble material	Elastin	
Rat*						
Aorta	317,300 ± 117,000	0.8	0.3	1.5	97.4 ± 2.3	7050 ± 210
Skin	5600 ± 940	n.d.†	2.3	49.6	48.3 ± 8.7	1800 ± 790
Tail tendon	1000 ± 260	n.d.†	48.7	19.3	32.3 ± 6.4	—
Dog‡						
Aorta	720,800	0.6	0.8	2.0	96.6	7200
Ligamentum nuchae	438,200	2.0	1.5	3.2	93.3	4300

* Data of rats are means of three animals.
† n.d., Not detectable.
‡ The dog was pretreated with a daily oral dose of 3 mg/kg non-labelled muzolimine for 5 weeks.

equivalent of *ca* 70 ng [^{14}C]muzolimine was bound to 1 mg aortic elastin.

Identification of elastin-bound radioactivity

The ^{14}C-radioactivity associated with the elastin of the arterial wall after administration of [^{14}C]muzolimine could not be extracted with 0.1 M NaOH or 0.1 M HCl or organic solvents, but was released by degradation of the elastin with pancreatic elastase (Fig. 1).

In the following, elastin preparations obtained from experimental animals pretreated with [^{14}C]-muzolimine or non-labelled muzolimine are designated '[^{14}C]muzolimine–elastin' or 'muzolimine-elastin', respectively.

Fractionation of the elastase-digested [^{14}C]muzolimine–elastin on HPLC resulted in a heterogeneous distribution of ^{14}C-radioactivity (Fig. 2A). When non-labelled elastin was degraded by pancreas elastase under identical conditions and [^{14}C]muzolimine was added at the end of the enzymatic reaction, a practically identical radioactivity pattern was obtained after HPLC separation (Fig. 2D) indicating a high affinity of [^{14}C]muzolimine to the elastin split products. When the elastase digest of [^{14}C]muzolimine–elastin was extracted with dichloromethane, more than 90% of the ^{14}C-radioactivity was detected in the organic phase and was found to have exactly the same retention time on HPLC (Fig. 2B) as

Table 2. Amino acid composition of elastin isolated from the aorta of rats which were on a standard diet (control) or on the same diet containing 800 ppm muzoline for 2 months (muzolimine)

Amino acid	Rat aortic elastins		Rat aortic elastin from ref. [26]
	Control	Muzolimine	
Hyp	14.7	17.6	18
Asx	8.3	9.5	8
Thr	19.2	20.2	21
Ser	17.7	17.1	14
Glx	18.0	18.6	15
Pro	94.3	99.6	104
Gly	370.2	351.2	377
Ala	209.5	201.0	196
Val	86.9	96.1	96
Ile	27.7	28.2	29
Leu	65.8	67.8	66
Tyr	30.5	30.1	30
Phe	17.4	19.1	13
Hyl*	1.3	2.4	—†
His	1.8	0.8	0.8
Lys	4.0	5.3	2
Arg	11.0	13.2	9
D+I‡	1.7	2.2	2

Values are means of an analysis in triplicate and are expressed as mole per 1000 mole. Reference values of rat aortic elastin are from ref. [26].
* Unidentified material occupying the elution position of hydroxylysine.
† Not determined.
‡ Refers to the sum of desmosine and isodesmosine.

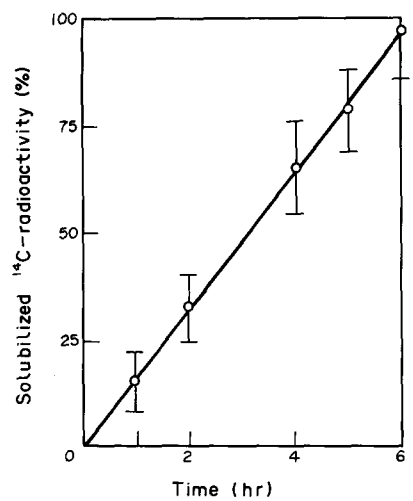

Fig. 1. Degradation of [^{14}C]muzolimine–elastin (sp. act. 7050 cpm/mg) by pancreatic elastase. 5 mg [^{14}C]muzolimine–elastin (particle size < 100 μm, see Materials and Methods) prepared from the aorta thoracica of rats pretreated with [^{14}C]muzolimine was preincubated in 2 ml of 0.1 m Tris–HCl buffer, pH 8.0, containing 2 mM $CaCl_2$ for 12 hr. After the addition of 18 U of elastase, 100 μl samples were withdrawn at the indicated time for radioactivity determination.

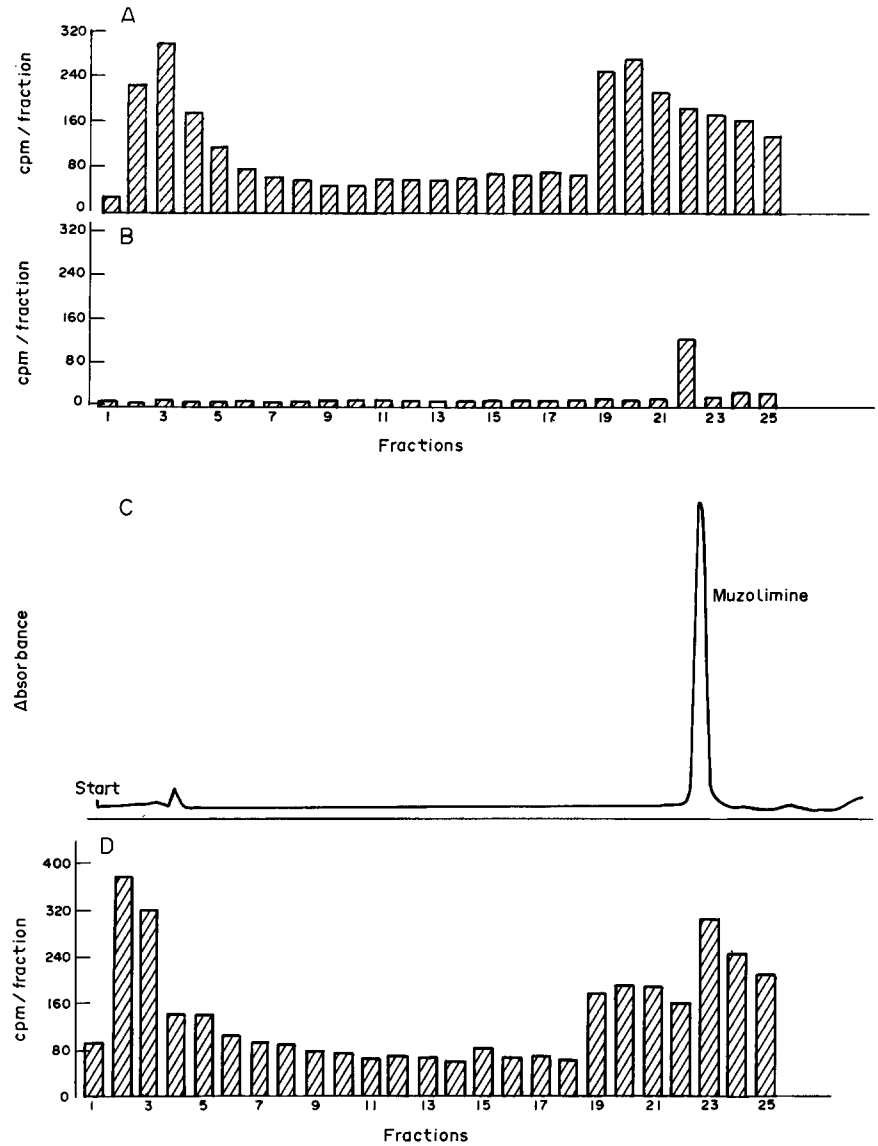

Fig. 2. (A) HPLC of an elastase digest of [^{14}C]muzolimine–elastin. Degradation conditions as in Fig. 1. 100 μl of the clear supernatant obtained after centrifugation of the incubation mixture was applied to the column. Radioactivity was plotted against retention time (one fraction/min). (B) HPLC of an elastase digest of [^{14}C]muzolimine–elastin. The digest was extracted with dichloromethane and the residue after evaporation of dichloromethane was redissolved in methanol and applied to the column. (C) Reference chromatogram of muzolimine. 10 μl of a 0.1% muzolimine solution in methanol was applied to the column and monitored by an UV detector (266 nm). (D) HPLC of an elastase digest of non-labelled elastin to which 10,000 cpm [^{14}C]muzolimine was added at the end of the enzymatic reaction (6 hr). Other conditions as in (A).

authentic muzolimine (Fig. 2C). The radioactivity fraction of Fig. 2B was collected, the solvent was evaporated under reduced pressure, and the residue analysed by mass spectrometry. The mass spectra unambiguously proved the presence of unchanged muzolimine extracted from the elastase digest as compared with authentic muzolimine.

The muzolimine content of muzolimine–elastin and [^{14}C]muzolimine–elastin of arterial tissue was determined by HPTLC. After long-term treatment of dogs with 1, 3 and 10 mg muzolimine per kg and day respectively, a dose-dependent binding of unchanged muzolimine to arterial tissue was observed. 1.9, 12.5 and 112.8 μg muzolimine/g wet wt of tissue corresponding to 4, 27 and 248 ng muzolimine/mg elastin were found.

An analysis of elastin-bound [^{14}C]muzolimine radioactivity on TLC plates by radioscanning revealed that part of the radioactivity remained at the origin of the chromatogram, giving evidence of muzolimine degradation products.

Influence of muzolimine on the elastin metabolism of aorta

In vitro incubation of aortic tissue under organ culture conditions in the presence of [^{3}H]glycine or

Table 3. Content of specific ^{3}H-radioactivity of aortic elastin and desmosine (D) and isodesmosine (I) isolated thereof

Group	Elastin (g/100 g dry wt)	Sp. radioact. (cpm/mg elastin)	D + I content of elastin (μg Lys equiv./mg elastin)	Sp. Radioact. of D + I (cpm/μg Lys Equiv.)
Control	44.6 ±3.1	7730 ±930	9.6 ±3.3	72.4 ±26.9
Muzolimine	45.4 ±2.2	7330 ±230	11.9 ±4.1	40.0 ±19.9
Significance vs. Control: P =	>0.1	>0.1	>0.1	<0.025

Elastin was prepared from rats on a standard diet or a muzolimine-containing diet for 2 months. The dissected aortas were incubated under organ culture conditions either in the presence of [2-^{3}H]glycine for labelling the elastin (column 2, $n = 12$) or in the presence of [4,5-^{3}H]lysine for labelling desmosine and isodesmosine (column 4, $n = 12$). D and I were isolated and assayed according to Starcher [11]. Values of D and I are expressed as lysine equivalents [27].

[^{3}H]lysine resulted in ^{3}H-labelling of elastin. When the aortas of rats were incubated under organ culture conditions for 5 days in the presence of [^{3}H]glycine, no differences in the content of elastin, in the amino acid composition of elastin, or in the specific radioactivity of the ^{3}H-labelled elastin could be detected between rats pretreated with muzolimine for 2 months and control animals. However, when [4,5-^{3}H]lysine was used as a label for the synthesis of elastin, the specific ^{3}H-radioactivity of desmosine and isodesmosine isolated from the elastin of muzolimine-pretreated animals was significantly lower than that of control animals (Table 3). The rate of solubilization by pancreatic elastase of ^{3}H-labelled elastin synthesized in organ culture and isolated from muzolimine-pretreated rats was enhanced, compared with ^{3}H-labelled elastin derived from control animals (Fig. 3A).

In contrast, over a longer time period, retardation of cross-linking of tropoelastin to elastin was not detectable as indicated by an equal desmosine and isodesmosine content of elastin in either animal group. However, the elastin-associated muzolimine suppressed the solubilization of elastin by pancreatic elastase (Fig. 3B).

DISCUSSION

After oral administration, [^{14}C]muzolimine enters the circulation and becomes closely associated with extracellular elastin of the arterial tissue and ligamentum nuchae. The [^{14}C]muzolimine-radioactivity bound to the elastin cannot be removed from the arterial tissue either by organic solvents or by treatment with 8 M urea or cyanogen bromide, but is released by the action of pancreatic elastase in soluble form and may be extracted from the elastase digest with dichloromethane.

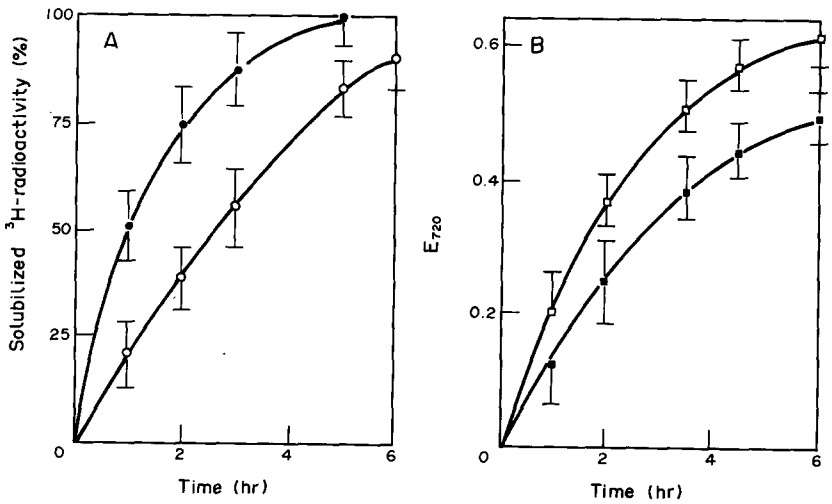

Fig. 3. (A) Degradation with elastase of [^{3}H]elastin prelabelled by incubation of rat aorta under organ culture conditions in the presence of [^{3}H]glycine. Preparation of equal-sized elastin particles and incubation conditions as in Fig. 1. Solubilized radioactivity was quantified. (○) Elastin prepared from the aorta of control animals; (●) elastin prepared from the aorta of animals pretreated with muzolimine for 2 months. (B) Degradation with elastase of non-labelled elastin isolated from normal rats (□, control) and from rats pretreated with muzolimine (■) for 2 months. Other conditions as in (A). The solubilized elastin split products were determined by the Lowry method.

Elastin has been reported to interact strongly with lipids [13] and lipophilic agents. Cholesterol ester [14, 15], fatty acids [15], sodium taurocholate [16] and elastophilic dyes (such as orcein [17]) are tightly bound to elastin, probably through bonding forces between lipophilic or aromatic groups and hydrophobic domains of the elastin molecule. In addition, forces other than those of hydrophobic binding may be involved.

The lipophilicity of muzolimine, which is shown by its octanol–water partition coefficient [18] and its solubility in non-polar organic solvents, explains the observed affinity of muzolimine to elastin. On the basis of an elastin content of total rat aorta (12 mg) and the amount of [^{14}C]muzolimine bound/mg aortic elastin, it is calculated that 0.04% of a single oral dose of 3 mg [^{14}C]muzolimine is incorporated into the aorta. In long-term experiments, the accumulation of muzolimine within the aorta of dogs increased with increasing time, reaching an amount of 250 ng muzolimine/mg aortic elastin after 52 weeks. This corresponds to a binding of *ca* 0.01% of the totally applied muzolimine dose (29.2 g) to the aorta thoracica and abdominalis.

The radioactivity and densitometric analysis of [^{14}C]muzolimine and non-labelled muzolimine incorporated into the aorta clearly proved the presence of unchanged muzolimine, but the existence of elastin-bound muzolimine derivatives cannot be excluded. However, since muzolimine is unstable under the conditions of the isolation procedure, it is not possible to determine whether the radioactivity present is attributed to metabolites or to non-enzymatically formed degradation products.

No systemic investigation on the turnover of elastin-bound muzolimine was undertaken. In a preliminary chase experiment (data not shown), no significant decrease of the [^{14}C]muzolimine radioactivity bound to aortic elastin during 10 days was observed. Studies on the question whether the muzolimine turnover parallels the turnover of aortic, are hampered by the fact that previous efforts to determine the turnover of elastin [19–22] have led to conflicting reports of the turnover time, ranging from a few weeks to years.

In vitro incubation experiments of rat aortic tissue in the presence of [^{3}H]glycine or [^{3}H]lysine gave evidence that muzolimine does not influence the intracellular biosynthesis of tropoelastin but retards the extracellular desmosine and isodesmosine formation. This assumption, however, is valid only for the *in vitro* incubation conditions used. Since measurements of elastin rely almost entirely on insoluble cross-linked elastin, the question of actual synthesis and secretion of soluble tropoelastin cannot be answered. Recent studies [23] have demonstrated that the tropoelastin synthesized in arterial wall cells is probably incorporated into elastic fibres without undergoing detectable proteolytic cleavage, and that the secretion of soluble tropoelastin and its incorporation into insoluble elastic fibres can be described by a single first-order process. The data of Kao *et al.* [23] are consistent with the hypothesis that elastic fibre growth occurs by the addition of individual tropoelastin molecules to existing fibres, and that the tropoelastin molecules are cross-linked to pre-existing elastic fibres by desmosine and isodesmosine formation and other covalent bonds. In the present experiments, only [^{3}H]desmosine and [^{3}H]isodesmosine were quantified. The possibility that other cross-links, such as lysinonorleucine, are increased in relation to desmosine and isodesmosine cannot be excluded.

From the results presented, it may be assumed that muzolimine bound to aortic elastin delays the process of tropoelastin incorporation into the extracellular muzolimine loaded-elastic fibres by suppressing the cross-linking reaction and the formation of desmosine and isodesmosine. This suggestion is derived from the lower specific radioactivity of [^{3}H]desmosine and [^{3}H]isodesmosine isolated from aortic elastin of rats pretreated with non-labelled muzolimine (Table 3) and from the lower proteolytic resistance of [^{3}H]glycine-labelled elastin isolated from the aorta of muzolimine-pretreated rats as compared with control animals (Fig. 3A). The results of Robert [24] and Mecham and Foster [25] have shown that elastin precursor molecules are extremely susceptible to a wide range of proteases and do not acquire the proteolytic resistance of mature elastin until fully cross-linked. However, since no changes in tissue concentration of elastin over a period of 2 months under muzolimine treatment were observed (Table 3), this effect seems to be of no importance for the *in vivo* elastin-forming processes. Especially as, on the other hand, results presented in Fig. 3B suggest a retarded degradation of muzolimine–elastin by pancreatic elastase. This finding is in contrast to the observation that an interaction between lipids and elastin leads to an accelerated degradation of elastin by elastase [13].

It seems conceivable from the present results that the formation of elastin under muzolimine treatment may be retarded, but once formed the elastin will be more resistant to elastase degradation. This effect might be of considerable value in the long-term treatment of cardiovascular disorders, some of which are known to be paralleled by an increased elastin degradation [13].

REFERENCES

1. D. Loew, W. Ritter and J. Dycka, *Eur. J. clin. Pharmac.* **12**, 341 (1977).
2. D. Schmidt, D. Loew, J. Dycka, H. Kopsa, P. Balcke, J. Zazgornik and E. Deutsch, *Eur. J. clin. Pharmac.* **14**, 399 (1978).
3. G. B. Ponti, M. Carnovali, A. Missaglia and G. Banderali, *Curr. theor. Res.* **28**, 520 (1980).
4. A. Dal Canton, D. Russo, R. Galbo, G. Conte and V. E. Andreucci, *Br. med. J.* **282**, 595 (1981).
5. H. Schiffl, P. Weidmann, C. Beretta-Piccoli, C. Cottier, A. J. Seiler and W. H. Ziegler, *Eur. J. clin. Pharmac.* **23**, 215 (1982).
6. W. Ritter, *Clin. Nephrol.* **19**, 26 (1983).
7. R. E. Chatelain, L. A. Ehrhart, P. M. Di Bello, B. N. Dardik and C. M. Ferrario, *J. Lab. clin. Med.* **97**, 700 (1981).
8. R. H. Cox, *Hypertension* **3**, 485 (1981).
9. B. L. Rasmussen, E. Bruenger and L. B. Sandberg, *Analyt. Biochem.* **64**, 255 (1975).
10. A. Schmidt, M. Prager, P. Selmke and E. Buddecke, *Eur. J. Biochem.* **125**, 95 (1982).
11. B. C. Starcher, *Analyt. Biochem.* **79**, 11 (1977).

12. W. Ritter, *J. Chromatogr.* **142**, 431 (1977).
13. L. Robert, in *Atherosclerosis V* (Eds. A. M. Gotto, L. C. Smith and B. Allen), p. 136. Springer, New York (1980).
14. B. Jacotot, J. L. Beaumont, G. Monnier, M. Szigeti, B. Robert and L. Robert, *Nutr. Metab.* **15**, 46 (1973).
15. M. Clair, B. Jacotot and L. Robert, *Connective Tissue Res.* **4**, 61 (1976).
16. A. M. Tamburro, V. Guantieri, D. Paga-Gordini and G. Abatangelo, *Biochim. biophys. Acta* **492**, 370 (1977).
17. L. A. Sachar, K. K. Winter, N. Sicher and S. Frankel, *Proc. Soc. exp. Biol. Med.* **90**, 323 (1955).
18. E. Möller, H. Horstmann and K. Meng, *Pharmatherapeutica* **1**, 540 (1977).
19. M. Lefevre and R. B. Rucker, *Biochim. biophys. Acta* **630**, 519 (1980).
20. G. M. Fischer and M. L. Swain, *Endocrinology* **102**, 92 (1978).
21. G. M. Fischer, *J. appl. Physiol.* **31**, 527 (1971).
22. R. L. Walford, P. K. Carter and R. B. Schnieder, *Archs Path.* **78**, 43 (1964).
23. W. W.-J. Kao, G. M. Bressan and P. J. Prockop, *Connective Tissue Res.* **10**, 263 (1982).
24. M. Robert, *Experientia* **30**, 211 (1974).
25. R. P. Mecham and J. A. Foster, *Biochemistry* **16**, 3825 (1978).
26. B. C. Starcher and M. J. Galione, *Analyt. Biochem.* **74**, 441 (1976).
27. I. A. Foster, *Meth. Enzym.* **82**, 559 (1982).