Platelet Aggregation Inhibiting and Anticoagulant Effects of Oligoamines, XV:

Antithrombotic Effect of Selected Oligoamines in Rats

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Oligoamines which exert antiplatelet and anticoagulant properties *in vitro* show as well antithrombotic effects in mesenteric arterioles and venoles of rats. The formation of thrombi in these vessels was induced by a laser beam and quantified by the thrombus formation index (TFI). The most potent compound RE 1492 already reduced the formation of thrombi after *i.v.* administration of 1 mg/kg significantly. After oral administration, however, only a *minor effect even after a 200 mg/kg dose is observed. This suggests that* the oligoamine was poorly absorbed from the gastrointestinal tract. The tricarbamate of RE 1492 (≡ RE 1492 C), however, was a suitable prodrug. Eight hours after a single oral dose of 10 mg/kg significant antithrombotic properties in arterioles and venoles were seen. (TFI = 3.63 (A), 1.77 (V); control: 1.76 (A), 1.29 (V).) After *p.o.* application of 30 mg/kg RE 1492 C the onset of activity is after 2 h (TFI = 3.44/1.48). A maximum effect is reached after 4 h (TFI: 4.43/2.84) and maintained up to 24 h (TFI = 4.49/2.45). After 48 h the effect in arterioles is still significant ($p < 0.05$, χ^2-test). The results obtained with five other carbamates (RE 2029 C, RE 1964 C, RE 2120 C, RE 2112 C, and RE 1981 C) 4 h after *p.o.* administration in general show a stronger effect in arterioles than in venoles which is in the same range as in RE 1492 C.

Antiaggregatorische und anticoagulante Eigenschaften von Oligoaminen, 15 Mitt.:
Antithrombotische Eigenschaften spezieller Oligoamine in Ratten

Oligoamine, die in vitro Hemmwirkung auf die Thrombocytenaggregation und anticoagulante Eigenschaften aufweisen, zeigen in Arteriolen von Ratten nach *i.v.* Gabe ab 1 mg/kg signifikante antithrombotische Wirkung. Diese wird durch eine verminderte Bildung von Thromben in Mesenterialarteriolen und -venolen von Ratten nach Laserbeschuß nachgewiesen. Nach oraler Gabe werden sie jedoch sehr schlecht resorbiert. Die korrespondierenden Carbamidsäureester erwiesen sich als geeignete Prodrugs. Das Tricarbamat von RE 1492 (*N,N',N''*-Tris-4-phenylbutyl-1,3,5-benzoltrimethanamin) ≡ RE 1492 C zeigte 8 h nach *p.o.* Gabe von 10 mg/kg sowohl in Arteriolen wie in Venolen signifikant antithrombotische Eigenschaften. Der Thrombusbildungsindex (TBI) betrug 3.63 bzw. 1.77 (*Kontrolle: 1.76 bzw. 1.29*). In Arteriolen hielt der Effekt bis 24 h nach Gabe an. Nach *p.o.* Verabreichung von 30 mg/kg setzt die Wirkung nach 2 h ein (TBI ≈ 3.44 bzw. 1.48), zeigt nach 4 h bereits das (in Arteriolen) erreichbare Maximum (TBI ≈ 4.43 bzw. 2.84). Die Wirkung hält dann bis 24 h an (TBI = 4.49 bzw. 2.45) und ist in Arteriolen noch nach 48 h signifikant ($p < 0.05$, χ^2-Test). Die mit den Carbamaten von fünf weiteren Oligoaminen (RE 2029 C, RE 1964 C, RE 2120 C, RE 2112 C und RE 1981 C) erhaltenen 4 h-Werte zeigen, daß die Wirkung in Arteriolen stets stärker ist als Venolen. Sie liegt im gleichen Bereich wie bei RE 1492 C und korrespondiert recht gut mit der *in vitro* gefundenen Hemmwirkung auf die Thrombocytenaggregation.

In a series of fourteen papers we have elucidated the structure activity relationships concerning the antiplatelet and anticoagulant activities of oligoamines. These properties could be demostrated *in vitro* in the *Born*-test and by the prolongation of the thromboplastin and partial thromboplastin time. The results gave strong evidence that these compounds could be able to develop antithrombotic efficacy. The latter can only be proved in an *in vivo* animal thrombosis model as all *in vitro* screens neglect the important blood fluidity parameter and the participation of the endothelial cells of blood vessels in the thrombotic process. We, therefore, have tested the most promising oligoamines in rats for antithrombotic activities. The scheme of the model we used is shown in fig. 1.

The formation of platelet thrombi in mesenteric arterioles and venoles of rats (diameter 15 µm) is induced with a laser beam by serveral shots of 50 ms and 50 mW. When 5 shots were insufficient to cause thrombus formation, this could not be reached even when a higher number of shots was applied. We, therefore, limited the number of shots to 5 in all our experiments. This model is rather expensive because an argon laser is needed. On the other hand, it is easy to handle and little surgical practice is required. The results are obtained quickly and are of good reproducibility. This model the idea of which seems to stem from *Arfors*[1], therefore, is widely accepted nowadays for the demonstration of antithrombotic properties of drugs[2-12].

The chemical structure of the compounds tested is shown in fig. 2.

The results obtained with RE 1492 and RE 1790 after intravenous injection are summarized in table 1 and compared with ASA, a widely used antiplatelet drug.

The oligoamine RE 1790 exerts significant antithrombotic properties in doses ≥ 3 mg/kg. The inhibition of thrombus formation is generally more evident in arterioles than in venoles. The same is true for RE 1492 which is more potent than RE 1790 and shows a significant effect in arterioles already in a 1 mg/kg dose. When a tenfold higher dose is administered nearly no thrombus formation takes place in the arterioles. Again the thrombus formation in venoles is

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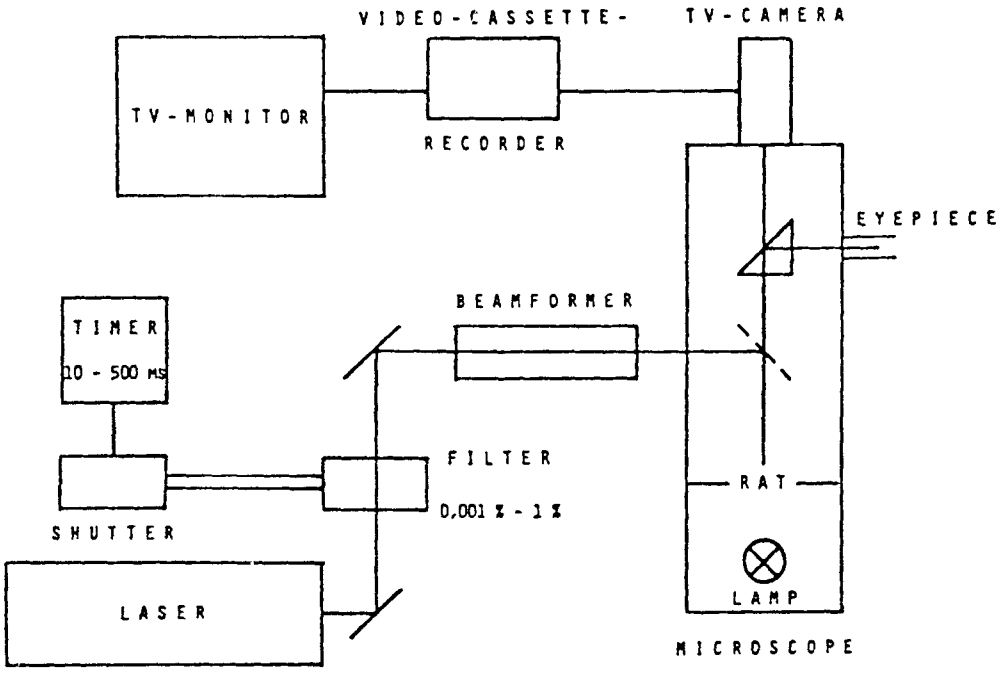

Fig. 1: Model for the demonstration of antithrombotic effects of drugs *in vivo*.

Table 1: Thrombus formation index (TFI) in mesenteric arterioles (A) and venoles (V) of rats 20 min after *i.v.* injection of acetylsalicylic acid (ASA), RE 1790, and RE 1492

Number of laser shots		1	2	3	4	5	>5	lesions	rats	TFI	s	χ^2	p<
Control	A	13	9	1	1	0	1	25	10	1.76	1.17	-	-
	V	25	10	0	0	0	0	35	6	1.29	0.46	-	-
ASA 30 mg/kg	A	16	12	5	3	1	7	44	6	2.59	1.80	3.01	0.1
	V	25	4	0	1	0	1	31	5	1.39	1.05	2.33	0.2
300 mg/kg	A	10	5	5	6	9	27	62	9	4.29	1.93	22.5	0.001
	V	8	3	1	2	3	9	26	6	3.61	2.19	24.46	0.001
RE 1790 1 mg/kg	A	28	10	2	2	2	5	49	5	2.08	1.68	1.60	-
	V	25	3	1	1	0	0	30	5	1.27	0.69	1.18	-
3 mg/kg	A	8	7	6	3	4	13	41	6	3.66	1.98	11.62	0.001
	V	27	4	5	1	0	5	42	6	2.00	1.68	5.42	0.05
10 mg/kg	A	11	5	4	1	4	19	44	5	3.89	2.17	14.71	0.001
	V	18	7	6	2	1	9	43	5	2.72	1.97	11.54	0.001
RE 1492 1 mg/kg	A	22	14	9	1	1	4	51	5	2.16	1.46	4.43	0.1
	V	35	5	3	0	0	0	43	5	1.26	0.58	0	-
3 mg/kg	A	13	11	4	0	3	13	44	6	3.18	2.11	6.65	0.01
	V	19	6	5	1	0	4	35	5	2.11	1.64	5.38	0.05
10 mg/kg	A	6	5	4	4	1	30	49	6	4.59	1.95	24.97	0.001
	V	8	6	4	0	3	8	29	5	3.28	2.07	16.03	0.001

inhibited to a smaller extent. With ASA similar effects can only be seen when a thirty-fold higher dose is used (300 mg/kg).

An antithrombotic drug has to be active after oral application especially when used for prophylactic purposes. The first two rows of table 2 show that unfortunately this is not the case with RE 1492. A TFI of 2.93 in arterioles after 200 mg/kg p.o. suggests that only 1% of this oligoamine is absorbed from the gastrointestinal tract. Therefore, a suitable prodrug had to be designed. It was found at last that the triethoxycarbonyl derivative of RE 1492 which was obtained by reaction of RE 1492 with the ethylester of chloroformic acid had the desired properties. As it is a tricarbamate it was named RE 1492 *C*. Such carbamides are known[13] to be stable against hydrolysis but are cleaved enzymatically *in vivo*. As this compound is a resinous liquid an o/w emulsion with olive oil and arabic gum was prepared.

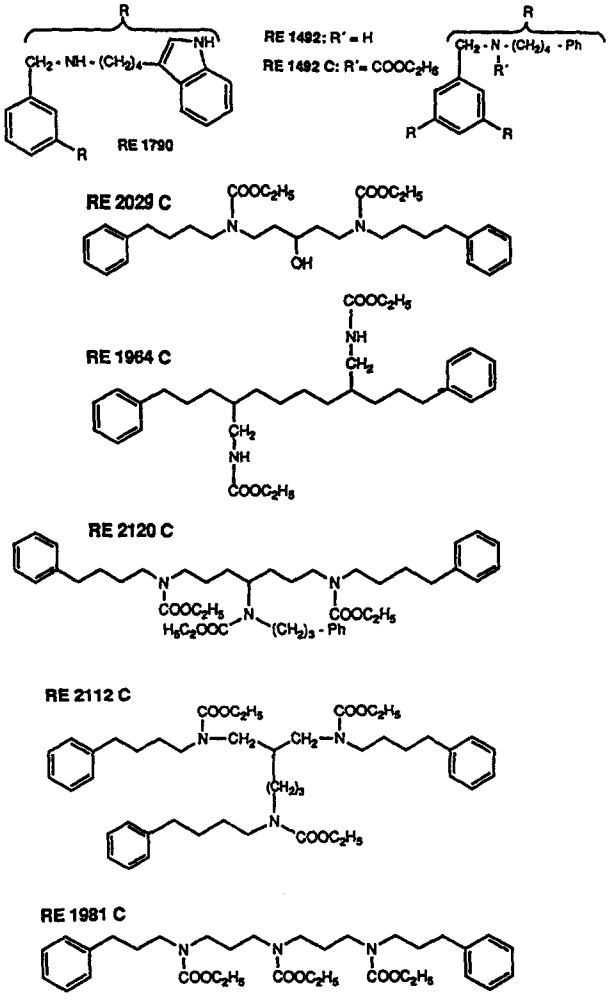

Fig. 2: Compounds tested for antithrombotic properties in rats by the device shown in fig. 1

The antithrombotic effects of RE 1492 C after *p.o.* administration are summarized in table 2.

8 h after a single oral dose of 10 mg/kg RE 1492 C a TFI of 3.63 in arterioles was observed. Even in venoles the antithrombotic effect was significant (TFI = 1.77). After 24 h still a significant inhibition of thrombus formation was observed suggesting a long duration of the drug effect. When 30 mg/kg RE 1492 C were given orally a maximum antithrombotic effect was already observed after 4 h (TFI = 4.33) which was maintained constant for about 20 h. Even 48 h after administration a significant effect was seen in arterioles (TFI = 2.98).

The results obtained with five other carbamate derivatives of oligoamines are summed up in table 3. RE 2029 was chosen as an example for a linear aliphatic diamine with a rather good solubility in water. RE 1964 is a prototype of a branched aliphatic diamine and yields the only secondary carbamate we have tested. RE 2112 and RE 2120 are representative for branched aliphatic triamines while RE 1981 is a candidate for linear aliphatic triamines with structural similarities to the naturally occuring triamine spermidine.

The antithrombotic effects only differ quantitatively from those obtained with RE 1492 C. In general TFI is higher in arterioles than in venoles. RE 2029 C, RE 1964 C, and RE 1981 C are of equal potency in arterioles while RE 2112 C and 2120 C appear to be somewhat weaker at a first glance. As their molecular weights are clearly higher this difference should not be overestimated. In venoles an effect significant on a 1%-level was only seen with RE 2029 C, RE 2112 C, and RE 1981 C. In summary, none of the oligoamines in table 3 was better than RE 1492 C. In general, all strong antiplatelet compounds tested so far in vivo as their carbamates developed similar antithrombotic effects. This seems to account only for minor differences in their pharmacokinetic behaviour.

Table 2: TFI in rats 2, 4, 8, 24 and 48 h after *p.o.* administration of RE 1492 and RE 1492 C.

Number of laser shots			1	2	3	4	5	>5	lesions	rats	TFI	s	χ^2	p<
RE 1492 p.o.														
200 mg/kg		A	23	7	7	2	1	15	55	5	2.93	2.11	5.60	0.05
		V	33	5	4	4	0	8	54	5	2.20	1.84	8.99	0.01
RE 1492 C p.o.														
10 mg/kg	8 h	A	9	11	11	4	3	18	56	5	3.63	1.92	10.44	0.01
		V	29	16	3	1	0	3	52	5	1.77	1.26	2.82	0.1
	24 h	A	9	9	9	7	2	10	46	5	3.30	1.80	8.63	0.01
		V	34	8	2	2	0	0	46	5	1.39	0.77	1.56	-
RE 1492 C p.o.														
30 mg/kg	2 h	A	13	13	10	6	2	19	63	6	3.44	1.96	9.84	0.01
		V	47	5	7	0	0	2	61	6	1.48	1.07	1.17	-
	4 h	A	4	7	6	3	2	25	47	5	4.43	1.89	20.60	0.001
		V	16	7	8	5	0	9	45	5	2.84	1.88	13.20	0.001
	24 h	A	6	5	6	3	3	28	51	6	4,49	1,91	23,16	0.001
		V	14	10	12	5	0	3	44	6	2.45	1.41	7.08	0.01
	48 h	A	21	11	6	3	2	15	58	6	2.98	2.06	6.29	0.05
		V	43	9	5	1	1	0	59	6	1.44	0.86	1.21	-

Table 3: TFI 4 h after oral administration of five oligoamine prodrugs (30 mg/kg)

Number of laser shots		1	2	3	4	5	>5	lesions	rats	TFI	s	χ^2	p<
RE 2029 C	A	6	8	5	7	5	18	49	5	4.04	1.87	19.11	0.001
	V	20	9	8	2	1	10	50	5	2.70	1.92	10.74	0.01
RE 1964 C	A	7	11	7	6	3	20	54	6	3.87	1.92	14.97	0.001
	V	34	9	5	2	0	1	51	6	1.59	1.04	2.13	0.2
RE 2112 C	A	18	10	3	6	1	16	54	6	3.19	2.10	9.45	0.01
	V	23	14	8	3	0	6	54	6	2.28	1.60	9.61	0.01
RE 2120 C	A	5	13	13	9	2	9	51	6	3.33	1.58	7.95	0.01
	V	41	7	3	1	0	1	53	6	1.40	0.93	1.35	-
RE 1981 C	A	7	9	8	4	1	22	51	6	3.96	1.98	14.36	0.001
	V	22	10	5	3	1	8	49	6	2.49	1.85	10.00	0.01

Experimental Part

Chemistry

Mp.: Mettler FP-1 (uncorrected), rise in temp. 2°/min.- Element analysis: Perkin-Elmer element analyzer 240 B and 240 C.- IR-spectra: Perkin-Elmer spectralphotometer 1420 with DS 7300.- ^{1}H-NMR-spectra: Bruker ACE 300 and WM 250.- Mass spectra: Varian MAT 711 (80 eV) and CH 7A (70 eV).- PI-FAB: Varian MAT CH 5 D*) DMSO/glycerol matrix.- Rotation chromatography: Chromatotron, Harrison Research, Palo Alto Cal.; sorbens: silicagel Merck 60 PF_{254}, art.-nr. 7749, thickness 4 mm; eluent ether or chloroform/ether 9:1 (RE 1964 C).

The synthesis of the oligoamines has already been described in former papers of this series. The desired carbamates were synthesized as follows:

To a stirred solution of 10 mmol of oligoamine in ether are added dropwise 10 mmol of ethyl chloroformate in ether for each amino function. The temp. is kept at 10°C. When half the ester has been added 10 mmol NaOH in 5 ml H_2O for each amino group are dropped in slowly. The solution is stirred for 1 h and the phases are separated. The ether phase is washed with water, dried over Na_2SO_4 and evaporated. Traces of ethyl chloroformate are removed in high vacuo. The crude product is purified by rotation chromatography.

N,N',N''-(α,α',α'')-Mesitylene-tris-N-4-phenylbutyl-carbamic acid ethylester (**RE 1492 C**)

Colorless, resinous liquid, yield 50%.- $C_{48}H_{63}N_3O_6$ (778.1) Calc. C 74.1 H 8.16 N 5.4 Found C 74.1 H 8.33 N 5.6.- IR ($CHCl_3$): 3003; 2932; 2860; 1688; 1604; 1474; 1424; 1386; 1191; 1163; 1117; 1026 cm^{-1}.- ^{1}H-NMR/250 MHz ([D_6]DMSO): δ (ppm) = 7.35-7.15 (m, 15H, arom.), 6.94 (bs, 3H, arom.), 4.39 (bs, 6H, Ar-C$\underline{H_2}$-N), 4.16 (q, J = 7 Hz, 6H, OCH_2), 3.18 (m, 6H, N-C$\underline{H_2}$-CH_2), 2.58 (t, J = 7 Hz, Ph-CH_2), 1.60 (m, 12H, CH_2-(C$\underline{H_2}$)$_2$-CH_2), 1.23 (t, J = 7 Hz, 9H, CH_3).- MS (240°): m/z = 778 (1%, M^+), 557 (38), 556 (100), 337 (20), 336 (24), 292 (10), 291 (23), 220 (30), 160 (13), 159 (10), 145 (12), 143 (11), 132 (12), 131 (50), 119 (53), 118 (36), 117 (33), 116 (14), 105 (17), 104 (10), 102 (16), 91 (64).

N,N'-(3-Hydroxypentane-1,5-diyl)-bis-N-4-phenylbutylcarbamic acid ethylester (**RE 2029 C**)

Colorless oil, yield 75%.- $C_{31}H_{46}N_2O_5$ (526.7) Calc. C 70.7 H 8.80 N 5.3 Found C 70.4 H 8.99 N 5.2.- IR (film): 3436; 2970; 2929; 2875; 1693; 1674; 1480; 1451; 1442; 1423; 1212; 770; 747; 699 cm^{-1}.- ^{1}H-NMR/300 MHz ($CDCl_3$): δ (ppm) = 7.30-7.15 (m, 10H, aromat.), 4.12 (q, J = 7 Hz, 4H, CH_2-O), 3.61 (m, 1H, OH), 3.47 (m, 1H, CH), 3.28 (m, 4H, N-C$\underline{H_2}$, CH_2-CH), 3.10 (m, 4H, N-C$\underline{H_2}$-$(CH_2)_3$), 2.63 (t, J = 7 Hz, 4H, CH_2-Ph), 1.59 (m, 12H, C$\underline{H_2}$-CH + CH_2-(C$\underline{H_2}$)$_2$-CH_2), 1.23 (t, J = 7 Hz, CH_3).- MS (80°): m/z = 526 (63%, M^+), 480 (43), 317 (33), 287 (28), 234 (74), 162 (31), 131 (44), 116 (48), 91 (100), 44 (41).

2,7-Bis-(3-phenylpropyl)-octane-1,8-dicarbamic acid ethylester (**RE 1964 C**)

Crystals, m.p. 94°, yield 65%.- $C_{32}H_{48}N_2O_4$ (524.8) Calc. C 73.3 H 9.22 N 5.3 Found C 73.2 H 9.22 N 5.3.- IR (KBr): 3341; 3055; 3019; 2975; 2926; 2853; 1691; 1602; 1540; 1493; 1453; 1373; 1271; 1249; 1149; 1030; 908; 777; 747; 698 cm^{-1}.- ^{1}H-NMR/250 MHz (*CDCl$_3$/CF$_3$COOD*): δ (ppm) = 7.3-7.16 (m, 10H, aromat.), 4.6 (m, 2H, -NH- exchange with D_2O), 4.1 (q, J = 7 Hz, 4H, O-CH_2), 3.8 (dt, J = 6/6 Hz, 4H, -NH-C$\underline{H_2}$, d after CF_3COOD), 2.6 (t, J = 7 Hz, 4H, Ph-CH_2), 1.6-1.2 (m, 24H, aliphat.).- MS (200°): m/z = 524 (30%, M^+), 478 (14), 131 (24), 117 (22), 104 (75), 102 (83), 91 (79), 74 (26), 30 (100).

N,N'-[2-(4-Phenylbutyl-N-ethoxycarbonyl-3-aminopropyl)-propane-1,3-diyl]-bis-N-4-phenylbutylcarbamic acid ethylester (**RE 2112 C**)

Colorless oil, yield 70%.- $C_{45}H_{65}N_3O_6$ (744.5) Calc. C 72.6 H 8.87 N 5.6 Found C 72.5 H 8.89 N 5.7.- IR (film): 2970; 2927; 2856; 1697; 1494; 1473; 1452; 1422; 1383; 1257; 770; 747; 700 cm^{-1}.- ^{1}H-NMR/300 MHz ($CDCl_3$): δ (ppm) = 7.29-7.14 (m, 15H, aromat.), 4.10 (q, J = 7 Hz, 6H, CH_2-O), 3.19-3.11 (m, 12H, CH_2-N), 2.62 (t, J = 7 Hz, 6H, CH_2-Ph), 1.64-1.56 (m, 17H, $(CH_2)_2$-CH + CH_2-(C$\underline{H_2}$)$_2$-CH_2), 1.21 (t, J = 7 Hz, 9H, CH_3).- MS (70°): m/z = 743 (77%, M^+), 670 (27), 523 (16), 509 (28), 449 (21), 301 (75), 234 (100), 131 (58), 91 (94), 44 (46).

1,7-Bis-[N-(4-phenylbutyl)ethoxycarbonylamino]-hept-4-yl-N-(3-phenylpropyl)-carbamic acid ethylester (**RE 2120 C**)

Colorless oil, yield 50%.- $C_{45}H_{65}N_3O_6$ (744.0) Calc. C 72.6 H 8.81 N 5.7 Found C 72.4 H 9.04 N 5.7.- IR (KBr): 3362; 3018; 2971; 2930; 2858; 2238; 1692; 1602; 1470; 1452; 1421; 1382; 1286; 1248; 1171; 1112; 1030; 891; 770; 748; 699 cm^{-1}.- ^{1}H-NMR/300 MHz ([D_6]DMSO): δ (ppm) = 7.29-7.16 (m, 15H, aromat.), 3.97 (m, 6H, O-CH_2), 3.35 (m, 1H, CH), 3.12-2.96 (m, 10H, N-CH_2), 2.55 (t, J = 7 Hz, 6H, Ph-CH_2), 1.77 (m, 2H, N-CH_2-C$\underline{H_2}$-CH_2-Ph), 1.46 (m, 8H, CH-(C$\underline{H_2}$)$_2$), 1.32 (m, 8H, Ph-CH_2-(C$\underline{H_2}$)$_2$), 1.12 (m, 9H, CH_3).- MS (70°): m/z = 743 (35%, M^+), 670 (39), 482 (30), 481 (84), 234 (28), 221 (50), 132 (25), 131 (43), 130 (29), 117 (21), 116 (30), 104 (28), 102 (54), 91 (100), 90 (20), 44 (27).

N,N-Bis-[3-[N-ethoxycarbonyl)-N-(3-phenylpropyl)-amino]-propyl]-cabamic acid ethylester (**RE 1981 C**)

Colorless oil, yield 80%.- $C_{33}H_{49}N_3O_3$ (583.7) Calc. C 67.9 H 8.46, N 7.2 Found C 67.7 H 8.64 N 7.2.- IR (KBr): 2972, 2926, 1698, 1473, 1452, 1422, 1383, 1293, 1244, 1174, 1117, 1062, 1028, 771, 748, 699 cm^{-1}.-

*) We thank *U. Ostwald* and *G. Holzmann* for measuring and discussing these mass spectra.

^{1}H-NMR/250 MHz ($CDCl_3$): δ (ppm) = 7.31-7.17 (m, 10H, aromat.), 4.11 (q, J = 7 Hz, 4H, CH_3-C<u>H_2</u>-O), 4.01 (q, J = 7 Hz, 2H, CH_3-C<u>H_2</u>-O), 3.22 (m, 12H, N-CH_2), 2.60 (t, J = 8 Hz, 4H, Ph-CH_2), 1.79 (tt, J = 8/8 Hz, 4H, N-CH_2-C<u>H_2</u>-CH_2-N), 1.74 (m, 4H, N-CH_2-C<u>H_2</u>-CH_2-Ph), 1.23 (t, J = 7 Hz, 6H, CH_3), 1.22 (t, J = 7 Hz, 3H, CH_3).- MS (400°): m/z = 584 (19%, M^+), 118 (31), 117 (25), 116 (45), 104 (33), 91 (55) , 78 (22), 70 (26), 57 (23), 56 (38), 44 (63), 43 (46), 42 (40), 41 (38), 30 (34), 29 (100).

Pharmacology

Apparatus

Laser: Coherent argon laser Innova 90-4 (all-line). Shutter, timer and beamformer: Dr *E. Unsöld*, GSF, Neuherberg.- Microscope: Olympus BHBJL with MD Plan 50.10 and MS Plan 500,60. The objectives must be heated to 40° to avoid condensation of water.- Cold light source: Heim L 100 with Xenon Lamp 100 N.- Constant temp. on the mesenterium: MGW Lauda, WB 20-512/12 with universal regulation box 2.- Power meter: Coherent Mod. 210.- Documentation: TV camera: Panasonic WV-1850/G with videoadapter Olympus MTV3 and intermediate objective Olympus 125 Fk 2,5x; Videorecorder: Sony U-matic VO-5630; TV-monitor; Electrohome type 38-V 19122-60; Camera: Olympus OM 2 with microadapter.- Sonification: Sonicator W 185 F Ultrasonics Inc.

Animal Experiments[*)]

Drug administration

Male Wistar rats weighing 120-150 g were kept without food 2 h before administration of the test compounds which are applied orally. For liquid compounds an emulsion was prepared by carefully grinding 2 parts (weight) arabic gum with 3 parts of water. Then a solution of 1.5 parts of the carbamic acid ester in 2 parts of olive oil is incorporated. Subsequently water is incorporated in small portions until altogether 30 parts in weight are reached. Final homogenization is performed by ultrasonification for 3 min. The emulsion has a drug content of 5%. The applied volume was 0.2-0.3 ml. The oligoamines RE 1492 and RE 1790 were dissolved in ehtanol/water (1:1). 100 µl were injected into the tail vene (see below).

Laser Thrombosis

The rats were anesthetized with a 1:1 mixture of Ketavet® (Ketamine · HCl 115 mg/ml) and Rompun (Xylazine · HCl 23.32 mg/ml) 0.1 ml/100 g body weight were injected intraperitoneally. After 5 min cornea reflexes are no longer observed. If the drug has to be administered i.v. this is performed now. In this case the preparation of the mesentery begins 15 min later. The mesentery is exposed by a hypogastric incision and spread flat on a self constructed object stage which was mounted on the microscope table. The mesentery is superfused with saline (37°C). Investigations were performed in arterioles (diameter: 10-25 µm) and venoles (10-30 µm) of the fat-free ileo-caecal portion of the mesentery. The laser beam was inserted in the light beam path of the microscope. The energy was adjusted to 50 mW at the object and controlled with the power meter. The exposure time to a single laser shot was 50 ms. The shot frequency was 30 s until a defined thrombus consisting of at least 10 platelets was formed. When no thrombus formation was observed after 5 shots the experiment at this lesion was finished. The next lesion was set either upstream in the same vessel or in another vessel so that an influence of the first lesion could be excluded. For one dose in at least 5 rats at least 40 lesions were induced. In one rat the number of lesions was not exceeding ten. At the end of the experiment the anesthetized rat was sacrificed by intrapulmonal injection of 0.3 ml T 61®.

For evaluation of the results the thrombus formation index (TFI) was calculated. It represents the average shot number necessary to form a thrombus. When no thrombus was formed after 5 shots a shot number of 6 was assumed. A TFI = 6, therefore, means that no thrombus formation at all could be observed. The significance of the results was checked by the χ^2-test.

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