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Original article

NO donors, part 8 [1]: synthesis and vasodilating activities of substituted benzylnitrates compared to cyclohexylmethylnitrate and GTN

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Abstract

A series of substituted benzylnitrates (**1**) and the formally but not chemically similar cyclohexylmethylnitrate (CHMN) have been synthesised. Vasodilating activities were measured on endothelium-intact and N^G-nitro-L-arginine (L-NNA)-blocked porcine right coronary arteries, precontracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Glyceroltrinitrate (GTN) was used as reference. In intact coronary arteries the vasodilating activities of all benzylnitrates are lower compared with GTN, but higher compared with CHMN. However, blocking the function of the endothelium by L-NNA, the activity of all benzylnitrates increased, whereas that of CHMN and GTN remained nearly unaffected. Under these conditions, the mononitrates 4-nitro-benzylnitrate (**1c**) and 4-nitrooxymethyl-benzonitrile (**1h**) even showed higher vasodilator activities than the trinitrate GTN and in general, vasorelaxation by the benzylnitrates as defined by the concentrations for half maximal effects (EC_{50} values) was found to be 2–3 orders of magnitude higher than that induced by CHMN. The study demonstrates that the in vitro activities of organic nitrates do not correlate with the number of nitrate groups within the molecule nor to the lipophilicity of the molecules. Instead, vasodilator activity is highly sensitive to the structure and the type of the substituents in the molecular carrier of the nitrate group.
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Keywords: Benzylnitrates; Organic nitrates; GTN; Vasodilator potency; Nitric oxide

1. Introduction

Organic nitrates have been used for the treatment of angina pectoris, acute myocardial infarction and acute as well as chronic congestive heart failure for over a century. Vasodilating activity and preload reduction, caused by these nitrates, are mainly attributed to the release of nitric oxide (NO), which is considered to be an enzymatic metabolic reduction process [2,3]. In contrast to that, recently evidence was found against NO being the active principle of glyceroltrinitrate (GTN) [4], which might revive the idea of a "nitrate receptor". In both cases, the vasodilator activity of an organic nitrate residue might be influenced—probably differently—by the chemistry of its molecular carrier and more structurally different nitrates should be pharmacologically examined.

Bearing this in mind, we synthesised a set of substituted benzylnitrates (**1**) and the formally but not chemically similar cyclohexylmethylnitrate (CHMN). Radicals may be intermediates in the degradation of organic nitrates and benzyl radicals are formed more easily than the cyclohexylmethyl radical. In addition, benzylnitrate (**1a**), but not CHMN, is known to undergo an internal redox reaction under elevated temperature yielding benzaldehyde and inorganic nitrite [5,6]. In order to evaluate the influence of the substituted benzyl structures on their vasodilating potency, we investigated the action of **1a**–**i**, CHMN and GTN as reference on endothelium-intact and N^G-nitro-L-arginine (L-NNA)-blocked porcine right coronary arteries, precontracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$).

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2. Chemistry

All of the organic nitrates **1a**–**i** and CHMN were prepared in satisfactory yield by treating the appropriate bromides with silver nitrate in dried acetonitrile (Fig. 1). If not available commercially, the benzylbromides **2** were obtained by bromination of the corresponding methylbenzenes with *N*-bromosuccimide (NBS). 4-Bromomethyl-benzaldehyde (**2d**) was synthesised by chemoselective reduction of 4-bromomethyl-benzonitrile (**2c**) with diisobutylaluminiumhydride (DIBAL-H) in chlorobenzene. The disubstituted benzylbromide **2e** was prepared by nitration of **5d** with KNO_3 dissolved in conc. H_2SO_4.

2.1. Stability

The stability of all organic nitrates was checked under simulated physiological conditions by dissolving 10 mg of the substances in a small amount of DMSO, then filling up to 10 mL with phosphate-buffer pH 7.4 and storing for 24 h at 37 °C. Before and after that period, GC-analyses using an Optima-5 column and an FID-detector were performed with 1 μL of the corresponding solutions. All substances could be detected without decomposition. Retention times (ranging from 4.2 to 7.9 min) and peak areas were identical before and after storing under these simulated physiological conditions.

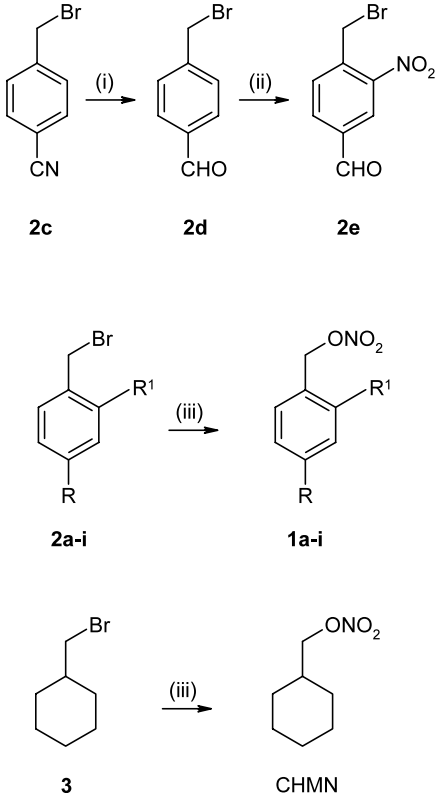

Fig. 1. Syntheses of nitrates and intermediates: (i) DIBAL, chlorobenzene, 0 °C; (ii) KNO_3, H_2SO_4, $CHCl_3$; (iii) $AgNO_3$, acetonitrile, r.t.

3. Pharmacology and discussion

All benzylnitrates as well as CHMN showed a dose-dependent vasorelaxation of porcine coronary arteries after precontraction with 50 μM $PGF_{2\alpha}$. Fig. 1 shows some relaxation curves, Table 1 gives the results of all compounds. GTN ($pD_2 = 6.46$) being the most active organic nitrate in intact porcine coronary arteries, CHMN displayed the lowest activity ($pD_2 = 3.80$) (Fig. 2). In between the benzylnitrates covering the full range of two orders of magnitude of pD_2 from 3.88 to 5.95.

In L-NNA-blocked vessels, CHMN remains the weakest vasodilator. While pD_2 values of CHMN and GTN changed only marginally, the benzylnitrates showed little (e.g., **1e**) or very significant (e.g., **1c**) increases of vasodilator activities (Fig. 3). In vessels with blocked endothelium, 4-cyano- (**1c**) and 4-nitro-substituted (**1h**) benzylnitrates showed an even higher vascular activity than GTN.

The influence of the endothelial function on the vasorelaxation by the organic nitrates can be expressed as the ratio (Q) of the concentrations for half maximal effects of the respective compounds in L-NNA-blocked coronary arteries versus endothelium-intact vessels. A rather strong influence of the endothelial function was observed with the benzylnitrates **1h**, **1f** and **1c** (Table 1, Fig. 3).

Our results give some indication for a high vasoactivity of benzylmononitrates as a new group of nitrovasodilators which are nearly equipotent to the trinitrate GTN. Differences in the efficacy of the compounds studied may be deduced from the different substituents. Rising the lipophilic properties of the compounds seems to decrease slightly rather than increase efficacy, however, the overall correlation of lipophilicity of the compounds studied (cf. Table 1) and the half maximal effects (given as pD_2 values, see Table 1) was not significant (Fig. 4). But up to now, any reliable correlations between electronic or steric parameters of the substituents and vasoactivity of the compounds could not yet be recognised. The higher efficacy of the benzylnitrates in L-NNA-blocked coronary arteries versus endothelium-intact vessels confirms former observations on nitrovasodilators, supporting a down-regulation of the soluble guanylcyclase as well as cGMP-dependent protein kinase by endogenous NO [7–9].

Moreover, CHMN causes the lowest activity in coronary arteries with intact as well as L-NNA-blocked endothelium, obviously the aromatic ring system generally enhances the liberation of NO or increases the

Table 1
Lipophilicity (log *P*) of and vasorelaxation (given as pD_2 values in $-\log \text{mol } l^{-1}$) by the benzylnitrates studied in $PGF_{2\alpha}$-precontracted intact porcine coronary arteries and after blockade by L-NNA

Compound	R	R^1	log *P*	Endothelium intact (increasing pD_2)	*n*	Compound	Endothelium blocked by L-NNA (increasing pD_2)	*n*	*Q* [a]
CHMN	–	–	–	3.80±0.03 [b]	8	CHMN	3.85±0.12 [b]	8	–
1f	t-C_4H_9	H	4.450	3.88±0.05	7	**1i**	5.02±0.29	8	1.04
1c	CN	H	2.057	4.08±0.38	7	**1g**	5.40±0.15	8	1.19
1g	CO_2H	H	2.367	4.52±0.08 [b]	8	**1f**	5.45±0.09	8	1.41
1i		H	2.240	4.85±0.09 [b]	7	**1d**	5.69±0.12	8	1.03
1h	NO_2	H	2.367	4.90±0.07	8	**1a**	5.86±0.14	8	1.09
1b	$CO_2C_2H_5$	H	3.122	5.16±0.09	8	**1b**	5.89±0.22	8	1.14
1a	H	H	2.624	5.36±0.29	8	**1e**	6.24±0.06	8	1.05
1d	CHO	H	1.977	5.51±0.01	8	GTN	6.33±0.13	8	0.98
1e	CHO	H	1.904	5.95±0.11	8	**1h**	6.59±0.31	8	1.34
GTN	–	NO_2	–	6.46±0.07	8	**1c**	6.74±0.06	8	1.65

Mean values and S.E.M. of *n* individual experiments are given.
[a] Ratio of pD_2 values of blocked to intact endothelium.
[b] Extrapolated values.

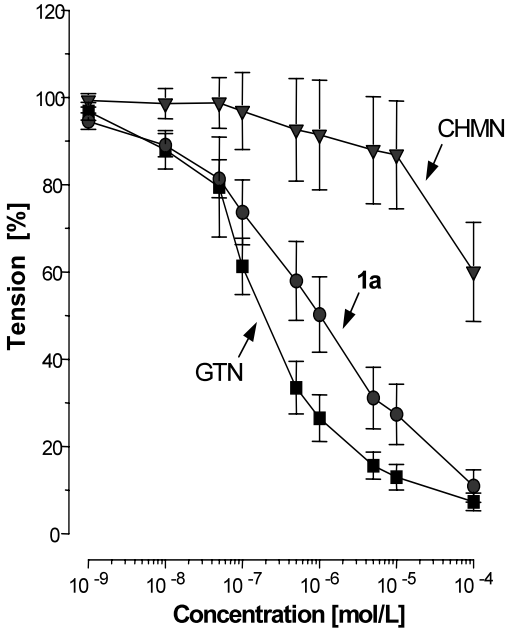

Fig. 2. Vasodilator activity of benzylnitrate (**1a**) [●] on isolated porcine right coronary arteries in comparison with CHMN [▼]. GTN [■] was used as reference.

affinity of the intact nitrate molecules to a putative nitrate receptor.

4. Experimental protocols

4.1. Chemistry

Melting points were determined in open capillary tubes on Gallenkamp melting point apparatus and are not corrected. IR spectra were recorded on a Perkin–Elmer 1420 or an FT-IR-spectrophotometer Paragon 1000 from Perkin–Elmer, respectively, using KBr pellets for solids and NaCl plates for liquid substances. ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectra were determined on a Bruker WH 90 (90 MHz) or a Bruker AC 200 (200 MHz) spectrometer, respectively, using $CDCl_3$ as the solvent. Chemical shifts are reported in δ (ppm) relative to tetramethylsilane as the internal standard. Elemental analysis indicated by the symbols of the elements were carried out with an Elementar Vario EL and were within 0.4% of the calculated values.

In general, organic nitrates are potential explosives. Due to the high CH:NO ratio, benzylmononitrates can be considered as relatively "safe" nitrates and the liquid compounds could be distilled without significant decomposition using the given conditions.

4.1.1. Ethyl 4-bromomethyl-benzoate (*2b*)

To a solution of 12.3 g (0.075 mol) ethyl 4-methylbenzoate in 75 mL of CCl_4 were added 13.3 g (0.075 mol) of NBS and 0.9 g of dibenzoylperoxide and the mixture was heated under reflux for 5 h. After cooling, the filtrate of the mixture was evaporated and the remaining oil was crystallised by trituration with Et_2O. The resulting solid was recrystallised twice from *n*-hexane. 10.25 g (56%) colourless crystals; m.p. 39–40 °C ([9], 41 °C); IR (cm^{-1}): 1730 (C=O); ^{1}H-NMR (90 MHz): $\delta = 1.33$ (t, $J = 7.1$ Hz, 3H, CH_3), 4.32 (q, $J = 7.1$ Hz, O–CH_2), 4.78 (s, 2H, CH_2–Br), 7.56 (d, $J = 8.3$ Hz, aromat. H-3, H-5), 7.95 (d, $J = 8.3$ Hz, aromat. H-2, H-6). Anal. ($C_{10}H_{12}BrO_2$): C, H, N.

4.1.2. 4-Bromomethyl-benzaldehyde (*2d*)

To a stirred solution of 6.0 g (30.6 mmol) of 4-bromomethyl-benzonitrile (**2c**) in 60 mL of dry chlor-

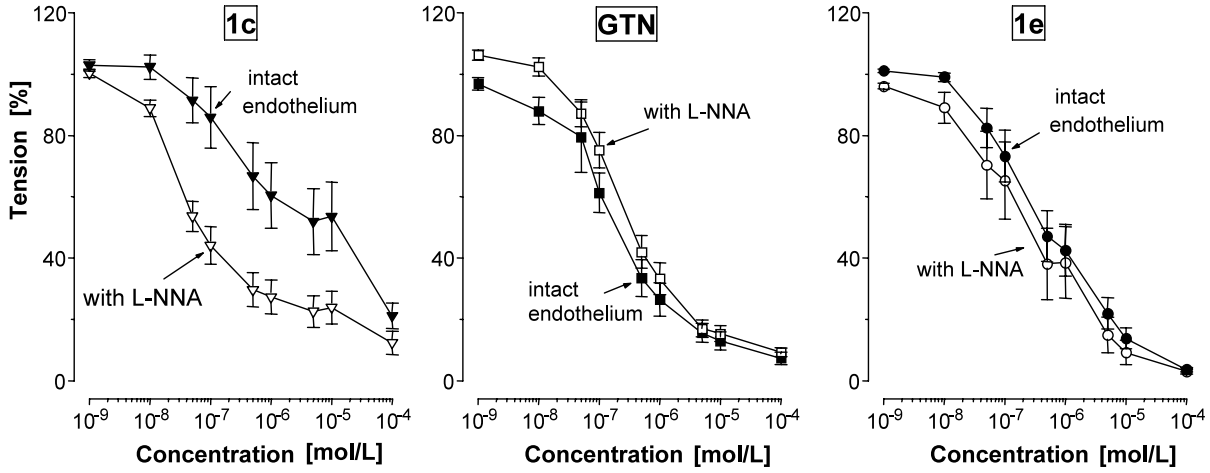

Fig. 3. Vasorelaxation by increasing concentrations of the benzylnitrates **1c** [▼/□] and **1e** [●/○] in comparison with GTN [■/□] in dependence on endothelial function (intact [▼●■] versus L-NNA-blocked vessels [□○□]).

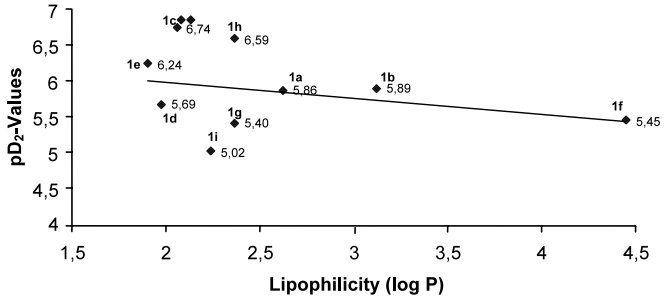

Fig. 4. Correlation between lipophilicity (log *P*) and the concentrations for half maximal effects (expressed as pD_2 values) of the organic nitrates in isolated porcine right coronary arteries.

obenzene, 50 mL of a 1 M solution of DIBAL-H in *n*-hexane was added over a period of 20 min at 0 °C. After stirring for 2.5 h at 0 °C the solution was diluted with 100 mL of $CHCl_3$, treated with 150 mL of an aqueous HCl solution (10%) and extracted into $CHCl_3$. The organic extracts were dried over Na_2SO_4 and evaporated to give an oil which was crystallised from Et_2O and recrystallised from *n*-hexane. 4.1 g (68%) pale yellow crystals; m.p. 96 °C ([10], 94–96 °C); IR (cm^{-1}): 1690 (C=O); ^{1}H-NMR (90 MHz): $\delta = 4.50$ (s, 2H, CH_2–Br), 7.53 (d, $J = 8.4$ Hz, 2H, aromat. H-3, H-5), 7.85 (d, $J =$ 8.4 Hz, 2H, aromat. H-2, H-6), 10.01 (s, 1H, CHO).

4.1.3. 4-Bromomethyl-3-nitrobenzaldehyde (*2e*)

To a solution of 1.4 g (0.014 mol) of KNO_3 in 15 mL of conc. H_2SO_4, a solution of 2.5 g (0.0125 mol) 4-bromomethyl-benzaldehyde (**2d**) in 10 mL of $CHCl_3$ was added dropwise at 0 °C. After stirring for 2 h at room temperature (r.t.), the mixture was poured into 500 mL of icewater. The precipitated crude product was separated by filtration, dried in vacuo and recrystallised from Et_2O to give **2e** as a yellow solid; 2.2 g (73%); IR (cm^{-1}): 1702 (C=O); ^{1}H-NMR (200 MHz): $\delta = 4.86$ (s, 2H, CH_2–Br), 7.76 (d, $J = 7.9$ Hz, 1H, aromat. H-5), 8.10 (dd, $J = 7.9/2.6$ Hz, 1H, aromat. H-6), 8.51 (d, $J =$ 2.6 Hz, 1H, aromat. H-2), 10.08 (s, 1H, CHO). Anal. ($C_8H_6BrNO_3$): C, H, N.

4.1.4. General procedure for preparation of benzylnitrates

A solution of benzylbromide **2** (15 mmol) in 30 mL of dry acetonitrile was added dropwise to a solution of 3.0 g (17.5 mmol) of $AgNO_3$ in 30 mL of acetonitrile and stirred at r.t. for 24 h. The filtrate of the mixture was poured into 800 mL of icewater. Precipitated solids were separated, dried in vacuo and recrystallised. Oils were extracted twice with 50 mL of CH_2Cl_2. The organic extracts were dried over Na_2SO_4, evaporated and the oily residue was distilled in vacuo.

4.1.5. Benzylnitrate (*1a*)

Colourless oil; 1.9 g (82%); b.p. 42 °C at 0.5 mbar ([11], b.p. 101–104 °C at 12 Torr); IR (cm^{-1}): 1633 and 1279 (N=O); ^{1}H-NMR (90 MHz): $\delta = 5.41$ (s, 2H, CH_2–ONO_2), 7.42 (s, 5H, aromat. H).

4.1.6. Ethyl 4-nitrooxymethyl-benzoate (*1b*)

Colourless crystals; 2.9 g (68%); m.p. 37–38 °C; IR (cm^{-1}): 1720 (C=O) 1640 and 1270 (N=O); ^{1}H-NMR (90 MHz): $\delta = 1.39$ (t, $J = 6.5$ Hz, 3H, CH_3), 4.38 (q, $J = 6.5$ Hz, 2H, O–CH_2), 5.43 (s, 2H, CH_2–ONO_2), 7.42 (d, $J = 7.8$ Hz, 2H, aromat. H-3, H-5), 8.10 (d, $J = 7.8$ Hz, 2H, aromat. H-2, H-6). Anal. ($C_{10}H_{11}NO_5$): C, H, N.

4.1.7. 4-Nitrooxymethyl-benzonitrile (*1c*)

White needles; 2.1 g (79%); m.p. 31 °C; IR (cm^{-1}): 2232 (C≡N) 1636 and 1282 (N=O); ^{1}H-NMR (90 MHz): $\delta = 5.48$ (s, 2H, CH_2–ONO_2), 7.50 (d, $J = 7.8$ Hz, 2H, aromat. H-3, H-5), 7.70 (d, $J = 7.8$ Hz, 2H, aromat. H-2, H-6). Anal. ($C_8H_6N_2O_3$): C, H, N.

4.1.8. 4-Nitrooxymethyl-benzaldehyde (**1d**)

Yellow needles; 1.2 g (45%); m.p. 43 °C ([10], 41 °C); IR (cm^{-1}): 1705 (C=O) 1650 and 1270 (N=O); ^{1}H-NMR (90 MHz): $\delta = 5.48$ (s, 2H, CH_2–ONO_2), 7.52 (d, $J = 7.6$ Hz, 2H, aromat. H-3, H-5), 7.93 (d, $J = 7.6$ Hz, 2H, aromat. H-2, H-6), 10.02 (s, 1H, CHO). Anal. ($C_8H_7NO_4$): C, H, N.

4.1.9. 3-Nitro-4-nitrooxymethyl-benzaldehyde (**1e**)

White needles; 2.7 g (81%); m.p. 62–64 °C; IR (cm^{-1}): 1711 (C=O) 1644 and 1284 (N=O); ^{1}H-NMR (200 MHz): $\delta = 5.96$ (s, 2H, CH_2–ONO_2), 7.81 (d, $J =$ 7.9 Hz, 1H, aromat. H-5), 8.21 (dd, $J = 7.9/2.6$ Hz, 1H, aromat. H-6), 8.66 (d, $J = 2.6$ Hz, 2H, aromat. H-2), 10.10 (s, 1H, CHO). Anal. ($C_8H_6N_2O_6$): C, H, N.

4.1.10. 4-tert-Butyl-benzylnitrate (**1f**)

Colourless fluid; 2.4 g (77%); b.p. 56 °C at 0.4 mbar; IR (cm^{-1}): 1633 and 1279 (N=O); ^{1}H-NMR (90 MHz): $\delta = 5.38$ (s, 2H, CH_2–ONO_2), 7.29 (d, $J = 9.0$ Hz, 2H, aromat. H-3, H-5), 7.45 (d, $J = 9.0$ Hz, 2H, arom. H-2, H-6); Anal. ($C_{11}H_{15}NO_3$): C, H, N.

4.1.11. 4-Nitrooxymethyl-benzoic acid (**1g**)

White powder; 2.45 g (83%); m.p. 164 °C ([12], 165 °C); IR (cm^{-1}): 1680 (C=O) 1640 and 1280 (N=O); ^{1}H-NMR (90 MHz): $\delta = 5.69$ (s, 2H, CH_2–ONO_2), 7.57 (d, $J = 8.1$ Hz, 2H, aromat. H-3, H-5), 7.99 (d, $J = 8.1$ Hz, 2H, aromat. H-2, H-6).

4.1.12. 4-Nitro-benzylnitrate (**1h**)

Colourless crystals; 2.4 g (82%); m.p. 66 °C ([13], 68.2 °C); IR (cm^{-1}): 1650 and 1281 (N=O); ^{1}H-NMR (90 MHz): $\delta = 5.72$ (s, 2H, CH_2–ONO_2); 7.70 (d, $J = 8.7$ Hz, 2H, aromat. H-2, H-6), 8.28 (d, $J = 8.7$ Hz, 2H, aromat. H-3, H-5). Anal. ($C_7H_6N_2O_5$): C, H, N.

4.1.13. 4-Nitrooxymethyl-benzaldehydeethylenacetale (**1i**)

A solution of 1.0 g (5.5 mmol) of 4-nitrooxymethyl-benzaldehyde (**1d**), 0.41 g (6.6 mmol) of ethylenglykole and 0.1 g of *p*-toloulsulfonic acid in 20 mL of $CHCl_3$ was refluxed for 48 h with continuous removal of water by a Dean-Stark apparatus. The solution was washed with an aqueous NaOH solution (10%) and the organic layer was dried over Na_2SO_4 and evaporated. The residue was recrystallised from MeOH to give a pale yellow solid; 2.26 g (70%); m.p. 42 °C; IR (cm^{-1}): 1638 and 1270 (N=O); ^{1}H-NMR (90 MHz): $\delta = 3.96$–4.15 (m, 4H, 2*x* O–CH_2), 5.41 (s, 2H, CH_2–ONO_2), 5.81 (s, 1H, O–CH–O), 7.38 (d, $J = 7.6$ Hz, 2H, aromat. 3-H, 5-H), 7.54 (d, 7.6 Hz, 2H, aromat. H-2, H-6). Anal. ($C_{10}H_{11}NO_5$): C, H, N.

4.1.14. Cyclohexylmethylnitrate

CHMN was prepared from cyclohexylmethylbromide (**3**) as described for the benzylnitrates; colourless fluid; 1.7 g (72%); b.p. 66 °C at 2.7 mbar; IR (cm^{-1}): 1635 and 1280 (N=O); ^{1}H-NMR (90 MHz): $\delta = 0.76$–2.15 (m, 11H, ring protons), 4.25 (d, $J = 6.5$, CH_2ONO_2). Anal. ($C_7H_{13}NO_3$): C, H, N.

4.2. Pharmacology

4.2.1. Vasodilator activity

Vasodilator activity was tested at a temperature of 37 °C on isolated right coronary arteries from porcine hearts which were obtained from a local slaughterhouse. Proximal parts of the arteries were rapidly dissected from the fresh hearts. Transport of the arteries was performed in a carbogen (95% O_2, 5% CO_2)-saturated Krebs–Henseleit solution of the following composition (in mmol L^{-1}): Na^+, 143.1; K^+, 5.9; Mg^{2+}, 16.0; Ca^{2+}, 1.6; Cl^-, 126.0; HCO_3^-, 25.0; H_2PO4^-, 1.2; SO_4^{2-}, 1.2; and glucose, 5.1; being equilibrated with carbogen to attain a pH of 7.4. The prepared arteries were cut into ring segments (3–4 mm), fixed between two stainless steel hooks as previously described [14] and incubated in a 10 mL organ bath containing equilibrated Krebs–Henseleit solution. Resting tension was 2 g. Vascular tone was measured isotonically using a strain gauge and was recorded after amplification (Fleck, Mainz, Germany) on a digital printing recorder (Linsseis, Selb, Germany). After an equilibration period of 45 min, during which the tissues were frequently washed, the arterial rings were contracted by 50 mmol L^{-1} $PGF_{2\alpha}$. After stabilisation, each compound studied was added in a cumulative manner at 30-min intervals to allow steady state of drug action. Each individual concentration–effect curve was followed by the addition of 0.2 mmol L^{-1} papaverine-HCl for maximal relaxation. For elimination of endothelial NO-synthase exposure to L-NNA (50 μmol l^{-1}) was allowed for 15 min (L-NNA-blocked vessels).

The contraction of the coronary arteries was calculated from the changes in their diameter using the equation $W = mgs$, with $W =$ work, $m =$ tension, $g =$ 9.81 m s^{-2}, $s =$ change in diameter, and is given in μJ. Vasorelaxation by the drugs studied is expressed as percentage of the maximal contractile response achieved with $PGF_{2\alpha}$ at the beginning of the experiments. The concentrations for half maximal inhibition of $PGF_{2\alpha}$-induced vasoconstriction (EC_{50} values) were calculated from the individual concentration–effect curves by logit transformation as proposed by Hafner et al. [15]. pD_2 values represent the negative logarithm of the concentration of the respective nitrates required for half maximal relaxation. All data were analysed by standard statistical methods (mean value and standard error of the mean, regression analysis, Student's *t*-test).

4.2.2. Materials

GTN was purchased from Merck, Darmstadt, Germany, $PGF_{2\alpha}$ (Minprostin® $F_{2\alpha}$ Amp) was a generous gift from Pharmacia & Upjohn, Erlangen (Germany). L-NNA was purchased from Sigma-Aldrich Chemie, Steinheim (Germany) and papaverine-HCl from Serva, Heidelberg (Germany).

All benzylnitrates were dissolved in DMSO, Merck, Darmstadt (Germany), all other chemicals in bidistillated water.

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