Synthesis and pharmacology of a series of new organic nitrate esters

J. Bron, G.J. Sterk, J.F. van der Werf and H. Timmerman

Introduction
The first organic nitrite and nitrate esters were discovered almost 150 years ago with the synthesis of amyl nitrite and glyceryl trinitrate (1) [1 2]. Since that time the clinically more important compound of these early investigations, glyceryl trinitrate, has been increasingly used for the treatment of patients with ischaemic heart diseases such as angina pectoris. Other nitrates, such as isosorbide dinitrate (2), erythritol tetrinitrate, mannitol hexanitrate and pentaerythritol tetrinitrate, were introduced only after 1940 [3 4]. Of these newer compounds, only isosorbide dinitrate and one of its active metabolites, isosorbide-5-mononitrate (3), gained considerable therapeutic importance. Presumably because of the continuing dispute about the occurrence of nitrate tolerance, the synthesis and pharmacology of newer nitrates have been largely neglected. Moreover, new chemical entities within other classes of cardiovascular agents, such as the calcium channel modulators and the angiotensin-converting enzyme inhibitors, have received overwhelming interest over the past decade because of their therapeutic potential.

The objective of our present investigations was to design new organic nitrate esters with haemodynamic profiles different from those of glyceryl trinitrate and isosorbide dinitrate, e.g. higher potency or strongly reduced induction of nitrate tolerance. In this paper we describe the synthesis of a series of new mononitrate and dinitrate esters and their pharmacology in vitro and in vivo. We attempted to establish qualitative structure-activity relationships for these new compounds.

Methods

Chemistry
The starting materials were at least synthesis grade as far as they were commercially available and they were purified when necessary. The reagents had a pro analyse quality and were obtained from Aldrich (Bornem, Belgium), Janssen Chimica (Geel, Belgium) and E. Merck Nederland (Amsterdam, the Netherlands). The new compounds were synthesized by nitroxylation according to the procedure described by Snatzke et al. [5] with minor modifications, as has been described elsewhere [6]. Briefly, 22 mmol of nitric acid were dissolved in 5 ml of glacial acetic acid and this mixture was added to a solution of 20 mmol of an alcohol (or 10 mmol of a diol), corresponding to the desired compound, in 50 ml of ethyl acetate at 0°C under nitrogen. After addition of 5 ml of acetic anhydride, the reaction mixture was kept at room temperature for 20 h and washed subsequently with a saturated solution of sodium hydroxide. The nitroxylated compounds were obtained following purification by chromatography [elution over a silica gel column with a mixture of petroleum ether (60-80°C) and diethyl ether] and/or crystallization. The purity and identity of the compounds were established by thin-layer chroma-
Pharmacology
Animal experiments were performed in accordance with the national laws on the care and use of laboratory animals, according to guidelines based on the licenses issued to the laboratories where the in vitro and in vivo experiments were carried out, namely the Department of Pharmacochemistry, Faculty of Chemistry, Free University, Amsterdam, the Netherlands, and the Department of Pharmacology, Faculty of Pharmacy, University of Utrecht, the Netherlands, respectively.

In vitro rat aorta assay
Male Wistar rats (210-240 g) were killed by decapitation and the heart was taken out together with the thoracic aorta by gently cutting the latter from the spine up to the diaphragm. The thoracic aorta (without aortic arch) was cut helically into strips 1-1.5 cm long and about 2 mm wide. These strips were placed in an organ bath (20 ml) containing Krebs-Ringer medium of the following composition (mmol/l): NaCl 118.5, KCl 4.74, MgSO₄ 1.18, KH₂PO₄ 1.18, CaCl₂ 2.5, NaHCO₃ 25, and glucose 10, bubbled with O₂ + CO₂ (95 + 5%) at 37°C. The preparations were allowed to stabilize for 100 min under a resting tension of 0.5 g. The buffer was replaced every 20 min. The strips were isotonically contracted with phenylephrine (10⁻⁷ mol/l). Drug-induced relaxation was tested at increasing concentrations (half log steps), until maximal or complete relaxation (corresponding to the basal precontraction of the organ) was reached. Responses were calculated as changes in organ length relative to the maximal displacement by contraction. EC50 values corresponded to the drug concentrations at which residual contractions were about 50% of the maximum.

Experiments with anaesthetized rabbits
New Zealand white rabbits (2.5-3 kg) were anaesthetized with pentobarbitone (30 mg/kg intravenously, supplemental doses as needed). Tracheotomy was performed and an intra-tracheal cannula was inserted. No artificial ventilation was applied and body temperature was maintained at 37-38°C, using an electric heating table. A Millar Mikro-Tip catheter was inserted via the right carotid artery, with the transducer in the left ventricle, for the measurement of left ventricular pressure. The left ventricular pressure signal was differentiated electronically to obtain the rate of change of left ventricular pressure (dP₀/dt). The heart rate was derived from the left ventricular pressure pulse signal. The jugular vein was cannulated for the infusion of test compounds. Aortic blood pressure (systolic and diastolic) was monitored (Gould-Statham pressure transducer) by inserting a polyethylene catheter filled with heparin (50 IU/ml) through the femoral artery into the abdominal aorta. Heparin (150 IU/kg, intravenously) was administered to prevent blood clotting. After completion of surgical procedures, the rabbits were allowed to recover for 15 min before drug administration was started.

The drugs were infused at a constant rate (0.5 ml/min) for 10 min at various doses, resulting in a typical dose range of 2.0, 20.0 and 200.0 µg·kg⁻¹·min⁻¹ (2.5 kg rabbit). Because of their poor solubility, all compounds tested were dissolved in dimethyl sulfoxide at 10 mg/ml and these solutions were further diluted with Intralipid (10%, Kabi Pharmacia, Woerden, the Netherlands), resulting in stock solutions of 1 mg/ml. Lower concentrations were obtained after dilution with the appropriate amount of Intralipid. Infusion of a control solution of 10% (v/v) dimethyl sulfoxide in Intralipid into the anaesthetized rabbit at a rate of 0.5 ml/min did not result in haemodynamic changes.

Log P calculations
The fragment constant of the nitrate ester (ONO₂) function was estimated. The log P values of the compounds were calculated using the hydrophobic fragmental values system [7]. The hydrophobic fragmental value for an ONO₂ group [(ONO₂)₃], used in these calculations, was derived from the estimated

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\begin{align*}
\text{Glyceryl trinitrate} & \quad \text{Isosorbide dinitrate} \\
\text{Isosorbide-5-mononitrate} & \quad \text{Isosorbide-5-mononitrate}
\end{align*}
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