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Effects of hypoxia on the vasodilator activity of nifedipine and evidence of secondary pharmacological properties

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Abstract

The effects of hypoxia on the vasodilator response of endothelium-denuded rat aortic rings to the calcium channel blocker, nifedipine, were examined. Under normoxic conditions, nifedipine (10^{-8}–3×10^{-6} M) attenuated the contractility of noradrenaline precontracted rings in a concentration-dependent manner, although the sensitivity was less than what occurs with K^+ precontracted tissues. Under hypoxic conditions there was no relaxation by nifedipine. When a concentration–response curve to noradrenaline was constructed before and in the presence of a high concentration of nifedipine (10^{-5} M), the response to noradrenaline was unaffected in both normoxic and hypoxic conditions. When noradrenaline was replaced by phenylephrine (10^{-8}–10^{-5} M), the maximum tension was reduced in the presence of nifedipine to 59±6% of the pre-nifedipine value. Repetition of the experiment in the presence of cocaine (10^{-5} M) revealed the inhibitory effect of nifedipine on noradrenaline-induced contraction, the maximum contraction in the presence of nifedipine falling significantly ($P<0.005$) to 67±6% of the pre-nifedipine response. When propranolol (10^{-7} M) was present in the bath, the maximum contraction to noradrenaline was significantly ($P<0.05$) reduced by nifedipine to 55±4% of its previous value. The fact that nifedipine was able to inhibit phenylephrine-induced contractions and relax noradrenaline-precontracted aortic rings confirms its calcium channel blocking activity. The failure to inhibit noradrenaline when added prior to the noradrenaline-induced contractions suggests an opposing effect in addition to calcium channel blockade, which cancels out the attenuation of noradrenaline — but not phenylephrine-induced contractions. When neuronal uptake of noradrenaline was blocked with cocaine or β-adrenoceptors were blocked with propranolol, the inhibitory effect of nifedipine against noradrenaline-induced contractions was revealed. This suggests that the additional property was due to blockade of neuronal reuptake or antagonism at β-adrenoceptors. This study also showed that nifedipine is ineffective as a vasodilator in the rat aorta under hypoxic conditions.

Keywords: Aorta; Nifedipine; Hypoxia; Noradrenaline; Phenylephrine

1. Introduction

Nifedipine is the prototype 1,4-dihydropyridine calcium channel blocker. By allosteric interference with the gating mechanism of L-type voltage activated calcium channels in smooth muscle, these drugs prevent the influx of extracellular calcium required to activate the contractile machinery of the cell (Godfraind, 1994; McDonald et al., 1994). Nifedipine exerts its clinical effects due to vasodilatation of arterial smooth muscle, leading to reduced peripheral resistance and improved coronary flow. It has little effect on cardiac tissue. Nifedipine is indicated for the prophylaxis of angina pectoris and in peripheral circulatory disorders such as Raynaud's syndrome (Godfraind, 1994).

Catecholamines such as noradrenaline and phenylephrine exert their contractile effects by their action on α-adrenoceptors. In the rat aorta, α_1-adrenoreceptors are predominant (Timmermans and Thoolen, 1987), specifically the α_{1D} subtype (Lyles et al., 1998). When activated, these receptors initiate a classic PLC-IP_3 mechanism leading to the release of calcium from stores in the sarcoplasmic reticulum (Hoffman and Taylor, 2001). It is likely that this causes a depolarisation of the cell membrane sufficient to activate voltage operated calcium channels (VOCCs) allowing calcium influx (Timmermans and Thoolen, 1987; Chen and Suzuki, 1989; Morel and Godfraind, 1991; Orallo, 1996; Gibson et al., 1998; Lyles et al., 1998; Hoffman and Taylor, 2001; McFadzean and Gibson, 2002; Ghisdal et al., 2003).

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The stimulation of α_1-adrenoceptors by noradrenaline (Godfraind, 1994) and phenylephrine (Delaflotte et al., 1989) therefore causes a biphasic contraction, comprising an initial fast component consistent with intracellular calcium release and a sustained tonic component when calcium enters the cell. Hence it is this tonic phase which is susceptible to perturbation by calcium channel blockers (Godfraind, 1976, 1988, 1994; Van Meel et al., 1981; Godfraind et al., 1982, 1986; Koch et al., 1990).

Some authorities have suggested a role for dihydropyridines in the acute treatment of ischaemic stroke. It has been demonstrated that the restoration of circulation to ischaemic areas is extremely important in minimising long term damage to areas of brain tissue and nifedipine may be expected to improve blood flow by its vasodilator properties. Such an action, however, would depend on nifedipine being effective under the hypoxic conditions prevailing in the circulation of the brain of the stroke victim (Kobayashi and Mori, 1998). Similarly nifedipine has been implicated for acute treatment in a number of other conditions involving impaired blood flow, these include peripheral vascular disease, intermittent claudication and the prevention of neurological damage during cardiac arrest (Triggle, 1997).

Several factors, however, limit the efficiency of calcium antagonists in increasing blood flow to ischaemic regions. For example, the hypotension caused by calcium channel antagonists may worsen the blood supply to the ischaemic area. Also, the blood vessels in the ischaemic region may already be dilated to their physiological maximum, hence the calcium antagonist dilates the blood vessels in other areas and the drug 'steals' blood flow to non-ischaemic regions.

The effectiveness of these proposed uses of calcium antagonists depends on the ability of these compounds to function under hypoxic or ischaemic conditions and surprisingly, there is very little published work on this topic. The evidence available is also conflicting. Herrera and Walker (1998) showed that in rat thoracic aortic rings contracted with phenylephrine or potassium, hypoxia causes relaxation, which is attributed to the blockade of Ca^{2+} channels because it is attenuated by the Ca^{2+} antagonist, nifedipine. Other evidence however shows that hypoxia causes Ca^{2+} influx and overload via L-type Ca^{2+} channels (Dixon et al., 1987). We have previously shown that the vasodilator response to adenosine is unaffected by hypoxia (Broadley and Maddock, 1996) and therefore it could not be predicted what effect hypoxia has on the actions of calcium channel antagonists.

The aim of this investigation was therefore to examine the effects of hypoxia on the vasodilator activity of nifedipine, with a view to assessing the likely usefulness of Ca^{2+} antagonists in ischaemic stroke. These experiments yielded unexpected results which led us to conduct experiments to identify secondary pharmacological properties of nifedipine.

2. Materials and methods

2.1. Drugs

The following drugs were supplied by Sigma (Poole, Dorset, U.K.): cocaine hydrochloride, nifedipine, (−)-noradrenaline bitartrate and phenylephrine hydrochloride. Ascorbic acid (10 μM) (B.D.H., Poole, Dorset, U.K.) was added to solutions to prevent oxidation of noradrenaline. (±)-Propranolol hydrochloride (AstraZeneca, Macclesfield, UK) was supplied as Inderal® injection (1 mg/ml).

All drugs were dissolved in double-distilled water with the exception of nifedipine which was dissolved in acetone (0.69 mg/ml) purchased from Fisons Scientific Equipment (Loughborough, U.K.) to form a stock solution. This was then diluted further using double-distilled water.

2.2. Tissue preparation

Animals were maintained in accordance with the Animals (Scientific Procedures) Act 1986. Male Wistar rats (250–350 g) were killed by a blow to the head and the thoracic aorta was removed. The aorta was gently rotated on a needle in order to denude the tissue of endothelium. Connective tissue was also gently removed. Sections of between 5 and 9 mm in length were set-up as ring preparations by suspending between two stainless steel hooks. The preparations were placed in a 20 ml organ bath at 37 °C in Kreb's solution (composition in mM: NaCl 118.4; $MgSO_4$ 1.2; KCl 4.7; $CaCl_2 \cdot 6H_2O$ 2.5; KH_2PO_4 1.2; $NaHCO_3$ 25.0 and glucose 11.7) and gassed with either 5% CO_2 in O_2 to simulate normoxic conditions or 5% CO_2 in N_2 to simulate hypoxia.

2.3. Measurement of contractile responses

The contractile tension of the tissue was measured via a Pioden dynamometer UF1 isometric transducer (range, ±55 g). The signal was amplified using Grass model 79D EEG polygraph data recording system (Grass instrument Co. Quincy, Mass., U.S.A.) / Maclab bridge amplifier and was converted from analogue to digital data and passed to a computer (Hardware: Powerlab 200 ADInstruments. Software: Chart v.4.1.1 sampling frequency 4 Hz). The apparatus was adjusted so that there was an initial resting tension of 1 g (±0.3 g). All preparations were left to equilibrate for 45 min and washed once with fresh Krebs' before any drugs were added to the bath.

2.4. Experimental protocols

2.4.1. Effects of nifedipine on noradrenaline precontracted aorta under hypoxic and normoxic conditions

Two tissues were taken from each animal and set-up in separate organ baths. After initial equilibration, both were subjected to a concentration–response curve to noradrenaline to confirm the viability of the preparation and to ensure a submaximal concentration of noradrenaline was used. The baths were then washed out and one bath was switched to hypoxic conditions and allowed to reach a stable baseline. Noradrenaline was then added to each bath to produce a concentration of 3×10^{-8} M and the tissues were left until the contractions had reached a stable plateau. A cumulative concentration–response curve to nifedipine was then obtained, commencing at 10^{-8} M and increasing the concentration in half-log intervals to a maximum of 3×10^{-6} M. Due to nifedipine