COMPARATIVE EFFECTS OF NIFLUMIC ACID ON NORADRENALINE-INDUCED PRESSOR RESPONSES IN VIVO AND IN VITRO IN THE RAT

(Efeitos comparativos do ácido niflúmico sobre as respostas pressoras da noradrenalina in vivo e in vitro em ratos)

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ABSTRACT

The effects of niflumic acid (NFA), a blocker of calcium-activated chloride channels (Cl\(_{\text{Ca}^-}\)), on noradrenaline (NA)-induced pressor responses were investigated in the isolated mesenteric vascular bed (MVB) and anaesthetized rat. In the MVB, in vitro pressor responses to 1 and 10 nmols of NA were inhibited concentration-dependently by NFA (10-30 M), with a maximum reduction of ~40% of the control, whereas KCl-induced responses were unaffected. In contrast in the anaesthetized rat, pressor responses to NA (1 mg/kg) were not significantly altered by NFA (1-30 mg/kg). Our results suggest that NFA exerts in vitro inhibitory effects on NA-induced vasoconstriction, however, this action does not manifest itself with respect to NA-induced increases in blood pressure in vivo.

KEY WORDS: niflumic acid, blood pressure, chloride, rat, mesenteric artery

RESUMO

Os efeitos do ácido niflúmico (NFA), um bloqueador dos canais de cloreto ativados pelo cálcio (Cl\(_{\text{Ca}^-}\)), foram investigados sobre as respostas pressoras da noradrenalina (NA) no leito mesentérico vascular (MVB) e no rato anestesiado. No MVB, as respostas pressoras in vitro de 1 e 10 nmols de NA foram inibidas de maneira dependente da concentração pelo NFA (10-30 M), enquanto as respostas induzidas por KCl não foram afetadas. Em contraste, no rato anestesiado, respostas pressoras da NA (1 mg/kg) não foram alteradas significativamente do NFA (1-30 mg/kg). Nossos resultados sugerem que o NFA exerce efeitos inibitórios in vitro sobre a vasoconstrição induzida por NA, no entanto, esta ação não se manifesta em relação aos aumentos de pressão arterial induzidos por NA in vivo.

PALAVRAS-CHAVE: ácido niflúmico, pressão vascular, cloridrato, rato, artéria mesentérica

INTRODUCTION

Neurotransmitters such as noradrenaline (NA) activate small conductance calcium-dependent chloride channels (Cl\(_{\text{Ca}^-}\)) in smooth muscle (for review see LARGE & WANG, 1996). Recent evidence has shown that niflumic acid (NFA) blocks IC\(_{\text{Ca}^-}\) in isolated smooth muscle cells from a variety of tissues (PACAUD et al., 1989; JANSSEN & SIMS, 1992; AKBARALI & GILES, 1993; HOGG et al., 1994; LAMB et al., 1994). We have previously shown that NA-induced contraction of the rat aorta is inhibited by niflumic acid (NFA), and have proposed that activation of Cl\(_{\text{Ca}^-}\) inducing depolarization and subsequent entry of calcium via voltage-dependent calcium channels...
(VDCCs), may be a mechanism utilized by NA to contract vascular smooth muscle (CRIDDLE et al., 1996). In a subsequent study we demonstrated that NFA exerts inhibitory effects on 5-HT and NA-induced pressor responses in the isolated mesenteric vascular bed of the rat (CRIDDLE et al., 1997). However, it is not known whether these inhibitory effects in vitro correspond to physiologically relevant actions in the whole animal.

The aim of the present study was to examine the actions of NFA on NA-induced pressor effects in the isolated mesenteric vascular bed (MVB) in vitro and on blood pressure in vivo in the anaesthetized rat.

METHODS

Mesenteric vascular bed

Male Wistar rats (250-350 g) were killed by stunning and exsanguination. The mesenteric vascular bed was dissected and mounted for perfusion via a modified method of MCGREGOR (1965) according to CRIDDLE et al. (1994). Briefly, preparations cannulated via the superior mesenteric artery were perfused at constant flow of 4 mL/min with a standard Tyrode’s solution of the following composition (mM): NaCl 136, KCl 5, MgCl\(_2\) 0.98, CaCl\(_2\) 2, NaH\(_2\)PO\(_4\) 0.36, NaHCO\(_3\) 11.9, glucose 5.5, at 37\(^\circ\)C and maintained at pH 7.4. Pressure changes were monitored with a mercury manometer and a pressure transducer connected to a computerized data acquisition system (Powerlab, AD Instruments).

NA (1 and 10 nmols) and KCl (20 mols) were applied as “bolus” (volume < 50 µL) injections directly into the perfusion stream. NFA (10-30 µM) was added to the reservoir at constant concentration.

Bolus injections of NA (1 and 10 nmols) elicited transient, reproducible rises in perfusion pressure of mean magnitude of 67.8 ±12.2 mmHg and 124.5 ± 6.3 mmHg, respectively. NA was then injected as a bolus of 1.5 nmol/kg until reproducible pressor responses were obtained. In order to evaluate the effects of NFA on these responses, NFA was administered as injections in doses of 0.3; 1.0 and 3.0 mg/kg 1 min before the injection of NA. Higher concentrations of NFA were not possible due to limitation of solubility.

Drugs

For in vitro experiments, niflumic acid (Sigma) was prepared as a stock solution in dimethyl sulphoxide (DMSO) and diluted in Tyrode’s solution on the day of the experiment. All drugs and reagents were purchased from Sigma Chemical Company.

Statistical analysis.

Data are expressed as the mean of n observations ± s.e. of mean. Inhibitory effects are expressed as % of control responses in the absence of the drug. Statistical analysis was performed using a paired or non-paired Student’s t-test where appropriate and values were taken to be significantly different when p < 0.05.

RESULTS

Effects of NFA on NA-induced pressor responses of the isolated MVB

Since niflumic acid has been shown to possess actions apart from blockade of ICl\(_{\text{Ca}}\) when in sufficient concentration, such as the induction of K\(^+\)-currents and blockade of Ca\(^{2+}\)-channels (HOGG et al., 1994; GREENWOOD & LARGE, 1995), we initially assessed the selectivity of this drug by evaluating its effects on KCl-induced pressor responses. In concentrations 30 µM, NFA did not inhibit the pressor effects of 20 nmols, however, a concentration of 100 µM reduced the response to 66.7 ± 18.3% of the control (n=8). Therefore when evaluating the effects of NFA on NA-induced responses only concentrations 30 µM were used.

Bolus injections of NA (1 and 10 nmols) elicited transient, reproducible rises in perfusion pressure of mean magnitude of 67.8 ±12.2 mmHg and 124.5 ± 6.3 mmHg, respectively.
A (10 and 30 µM) inhibited the pressor responses induced by 1 and 10 nmols NA in a concentration-dependent manner (Fig. 1, n =13), with a slightly greater inhibition of the lower dose of NA occurring with 30 µM NFA (64.4 ± 6.4 % of the control value).

Effects of NFA on NA-induced pressor effects in the anaesthetized rat

The mean blood pressure of the rat experimental group was 63.0 ± 5.1 mmHg (n=7). Injection of NA (1.5 mg/kg) induced transient rises in blood pressure of 16.4 ± 1.7 mmHg.

Figure 1. Inhibitory effects of niflumic acid (10 and 30 µM) on 1 and 10 nmols noradrenaline (NA)-induced increases in perfusion pressure in the mesenteric vascular bed of the rat. (Values are shown as the mean ± s.e. of mean of 13 experiments, and are shown to differ significantly from the control when p < 0.05 *).

Figure 2. Effects of niflumic acid (0.3 – 3.0 mg/kg) on transient increases in blood pressure induced by bolus injections of noradrenaline (1.5 mg/kg) in the anaesthetized rat. (Values are shown as the mean ± s.e. of mean of 7 experiments, and are shown to differ significantly from the control when p < 0.05).
(n=7) that were reproducible throughout the experimental period. NFA did not significantly inhibit the pressor effect of NA, the responses being 108.2 ± 8.8%, 105.4 ± 12.7% and 85.4 ± 15.1% in the presence of 0.3, 1.0 and 3.0 mg/kg, respectively (Fig. 2, n=7).

**DISCUSSION**

The present study has confirmed that NFA inhibits NA-induced pressor effects in the isolated mesenteric vascular bed of the rat, supporting previous observations in this preparation (CRIDDLE et al., 1997) and in the isolated rat aorta (CRIDDLE et al., 1996). Since the concentrations of NFA used in our present study correspond to those previously shown to inhibit $\text{ICl}_{Ca}$ in isolated smooth muscle cells (PACAUD et al., 1989; JANSSEN & SIMS, 1992; AKBARALI & GILES, 1993; HOGG et al., 1994; LAMB et al., 1994), our results appear to support the current opinion that activation of calcium-dependent chloride currents is a mechanism whereby NA may induce contraction of blood vessels (LARGE & WANG, 1996). We have previously suggested that this mechanism may lead to an entry of extracellular calcium ions via voltage-dependent calcium channels thus eliciting the contractile response of NA (CRIDDLE et al., 1996) and recently several studies in whole tissues and single cells have indicated that NA and other neurotransmitters may share this common excitatory pathway (GUIBERT et al., 1997; YUAN, 1997; HYVELIN et al., 1998; LAMB & BARNA, 1998). However, these in vitro studies have not addressed a putative physiological relevance of activation of $\text{ICl}_{Ca}$ to the pressor effects of neurotransmitters in vivo.

Our present data suggest that intravenous administration of NFA is not able to inhibit the pressor effects of NA in the anaesthetized rat at the doses tested. The highest dose (3 mg/kg) of NFA employed in our study showed a tendency to reduce the increase in mean arterial pressure induced by the bolus dose of NA, however, this was not significant. Unfortunately due to limitations of solubility larger doses of NFA could not be used, leaving open the possibility that higher doses may still exert significant inhibitory effects on NA-induced pressor responses. Our present in vivo data are in accord with a previous study, that failed to demonstrate an inhibitory action of NFA (up to 3 mg/kg) on cirazoline-induced increases in blood pressure in the anaesthetized rat (HE & TABRIZCHI, 1997). However, HE & TABRIZCHI (1997) also found that local administration of NFA inhibited decreases in vascular conductance induced by cirazoline in the mesenteric vasculature in vivo in similar doses. This may indicate that in the present study, when administered systemically, NFA might not reach an effective concentration sufficient to reduce NA-induced increases in mean arterial blood pressure. A simple dilution of NFA within the systemic circulation may be responsible for this, however, many other factors such as drug metabolism and protein binding may influence the outcome.

In conclusion, we have shown that NFA does not inhibit NA-induced pressor effects in the anaesthetized rat in vivo, despite exerting such effects in the in vitro mesenteric vascular bed. This apparent discrepancy may be due to an insufficient effective concentration of NFA reaching its site of action when administered intravenously.

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**REFERENCES**


