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European Journal of Pharmacology 406 (2000) 247–255

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Differences in the nitric oxide/soluble guanylyl cyclase signalling pathway in the myocardium of neonatal and adult rats

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Received 15 May 2000; received in revised form 18 August 2000; accepted 22 August 2000

Abstract

The effects of a nitric oxide-donor, *S*-nitroso-*N*-acetylpenicillamine, and a direct activator of soluble guanylyl cyclase, 3-(5′-hydroxymethyl-2′-furyl)-1-benzyl indazole (YC-1), on force of contraction (F_c) and L-type Ca^{2+} currents ($I_{Ca(L)}$) were investigated in myocardial preparations from neonatal and adult rats. Since hearts from adult and neonatal animals contained 160 and 47 mg/100 g wet weight myoglobin, respectively, its possible interaction with both drugs was also investigated. Both *S*-nitroso-*N*-acetylpenicillamine (100 μM) and YC-1 (30 μM) were ineffective in myocardial preparations from adult rats but reduced the magnitude of $I_{Ca(L)}$ and F_c in preparations from neonatal rats. The latter effects were antagonised by 1*H*-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ; 50 μM) and attenuated by myoglobin (30–300 μM), which also attenuated the effects of both drugs on pre-contracted aortic rings. The differential effects of *S*-nitroso-*N*-acetylpenicillamine and YC-1 in the myocardium from adult and neonatal rats may result from developmental changes in the content of myoglobin and/or in the NO/soluble guanylyl cyclase signal pathway.

Keywords: Ventricular cardiomyocyte, rat; Contractile function; Ca^{2+} channel, L-type; Nitric oxide (NO); Myoglobin

1. Introduction

It is well established that nitric oxide (NO) plays an important role as a signal molecule in several physiological processes, including regulation of vascular tone, inhibition of platelet aggregation, and synaptic transmission (Moncada et al., 1991; Garthwaite, 1995). The second messenger transmitting the effects of NO is usually cGMP, NO being a powerful activator of soluble guanylyl cyclase (Murad, 1994).

Although evidence is accumulating that cardiac NO production is increased under cardiomyopathic conditions (Finkel et al., 1992; Ungureanu-Longrois et al., 1995), the effects of NO on myocardial contractility are still a matter of debate. For instance, in various cardiac tissues, NO or related substances have been found either to reduce (Smith et al., 1991; Brady et al., 1992; Flesch et al., 1997) or to have no effect on cardiac contractility (Weyrich et al., 1994; Nawrath et al., 1995). Even positive and negative inotropic effects of NO-related substances have been reported in frog cardiomyocytes (Méry et al., 1993) and canine papillary muscle (Mohan et al., 1996), dependent on the concentration used.

The cardiac effects of NO have been commonly ascribed to activation of soluble guanylyl cyclase followed by an increase in cGMP levels (Hare and Colucci, 1995). However, given the broad chemistry of NO and its redox-activated forms (NO^- and NO^+), it is not surprising that this molecule can affect a number of proteins besides soluble guanylyl cyclase (Stamler et al., 1992), which may account for the observed differences in the cardiac effects of NO. For example, Ca^{2+}-dependent K^+-channels have been shown to be directly activated by NO in vascular smooth muscle (Bolotina et al., 1994). Ca^{2+} channels and their α-subunits have been reported to be inhibited by the NO-donor *S*-nitroso-*N*-acetylpenicillamine independent of cGMP in tsA201 cells, probably by sulfhydryl-nitrosylation of the protein (Campbell et al., 1996; Bernatchez et al., 1999).

The introduction of substances which activate soluble guanylyl cyclase independent of NO (like 3-(5′-hydroxymethyl-2′-furyl)-1-benzyl indazole (YC-1); Wu et al., 1995) or which inhibit selectively soluble guanylyl cyclase (like

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0014-2999/00/$ - see front matter
PII: S0014-2999(00)00654-3

1*H*-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ); Garthwaite et al., 1995) was expected to clarify some of these discrepancies. ODQ antagonised the inhibitory effect of *S*-nitroso-*N*-acetylpenicillamine on the isoprenaline-stimulated L-type Ca^{2+} current ($I_{Ca(L)}$) in frog cardiomyocytes (Abi-Gerges et al., 1997). However, ODQ did not attenuate the increase in cGMP levels induced by *S*-nitroso-*N*-acetylpenicillamine in isolated rat cardiomyocytes (Wegener et al., 1999). In addition, YC-1 was ineffective on force of contraction (F_c), $I_{Ca(L)}$, and cGMP levels in the rat myocardium (Wegener et al., 1997).

Recently, perinatal changes in the expression of soluble guanylyl cyclase and of a NO synthase isoform have been reported in rat pulmonary tissue (Bloch et al., 1997) as well as in murine myocardium (Ji et al., 1999), indicating that the role of the NO/soluble guanylyl cyclase/cGMP signalling pathway decreases during maturation. In the present study, the effects of the NO-donor *S*-nitroso-*N*-acetylpenicillamine and of the direct activator of soluble guanylyl cyclase YC-1 on F_c and $I_{Ca(L)}$ were, therefore, studied in heart muscle preparations from neonatal and adult rats. In addition, the effects of myoglobin, which possibly acts as a scavenger of both NO and YC-1, were investigated on *S*-nitroso-*N*-acetylpenicillamine- and YC-1-induced relaxation in pre-contracted aortic rings. A preliminary account of this work has been presented (Vulcu et al., 2000).

2. Methods

2.1. Preparations

Adult Sprague–Dawley rats (200–300 g) of either sex were anaesthetised with ether and bled from the carotid arteries. The heart and the thoracic aorta were quickly removed and immersed in warmed and oxygenated Tyrode's solution (containing in mM: NaCl 137, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1, $NaHCO_3$ 12, NaH_2PO_4 0.42, glucose 5.6; bubbled with 95% O_2 + 5% CO_2; pH 7.4). Cardiac muscle strips were prepared from the right ventricle and supplied at either end with silk ligatures. The aorta was cut into rings of 3–5 mm through which silk ligatures were threaded. Single ventricular cardiomyocytes were isolated as described previously (Wegener and Nawrath, 1995). Briefly, the hearts were enzymatically digested by perfusion with a collagenase-containing buffer solution via the aorta using the Langendorff set-up. Single myocytes were obtained from ventricular tissue pieces by mechanical dispersion.

Neonatal Sprague–Dawley rats were killed by cervical dislocation on the day of birth. The heart was quickly removed and immersed in warmed and oxygenated Tyrode's solution. Muscle strips were prepared from both ventricles and supplied at either end with silk ligatures. For the isolation of single ventricular cardiomyocytes, heart pieces were transferred to a nominally Ca^{2+}-free buffer solution (containing in mM: NaCl 135, KCl 5.4, KH_2PO_4 1.2, $MgCl_2$ 1, glucose 30, HEPES 10; pH was adjusted to 7.4 with NaOH) to which collagenase A (1.8 units/10 ml) was added. After about 45–60 min, the ventricular tissue pieces were mechanically dispersed in a freshly prepared nominally Ca^{2+}-free solution until a sufficient number of single cells were released. The isolated cells were stored at 8°C and used for electrophysiological experiments within 4 h.

The experiments conformed with the Guide for the Care and Use of Laboratory Animals (www.nap.edu/readingroom/books/labrats/).

2.2. Measurement of tension

Cardiac ventricular strips or aortic rings were mounted vertically in organ baths (5 ml) containing oxygenated Tyrode's solution at 36 ± 1°C. One end was fixed to a hook of a muscle holder while the other end was connected to an inductive force-displacement transducer whose output was fed to a carrier frequency preamplifier (Carrier amplifier/TA2000, Gould, Cleveland, OH, USA). The cardiac preparations were stretched to the apex of the preload active tension curve. Aortic rings were stretched by about 5 mN and then pre-contracted with phenylephrine (3 μM). Cardiac muscle strips were mounted next to two platinum electrodes built in a muscle holder and electrically stimulated by square-wave voltage pulses at 3 Hz (Grass S4, 1 ms duration, voltage 20% above threshold). Drugs were added from stock solutions to the organ bath as single or repeatedly applied doses to achieve the final concentrations indicated.

2.3. Measurement of $I_{Ca(L)}$

Electrophysiological experiments were performed on myocytes with clear cross striations using the whole-cell configuration of the patch-clamp technique (Hamill et al., 1981). The experimental equipment used has been described elsewhere (Wegener and Nawrath, 1995). During the experiments, the myocytes were voltage-clamped at a holding potential of −80 mV. To inactivate the fast sodium current, a 20-ms pre-pulse to −40 mV was set before activation of the Ca^{2+}-current. $I_{Ca(L)}$ were elicited by 100-ms depolarising voltage pulses to 0 mV at 0.2 Hz. The experiments were performed at 36 ± 1°C.

2.4. Determination of the myoglobin content

The myoglobin content of the cardiac tissue was determined according to O'Brien et al. (1992). Briefly, tertiary-butyl hydroperoxide and orthotolidine were used to generate a blue colour with the haem group of myoglobin. The absorbance was monitored at 630 nM and compared with standards. To avoid contamination with haemoglobin de-

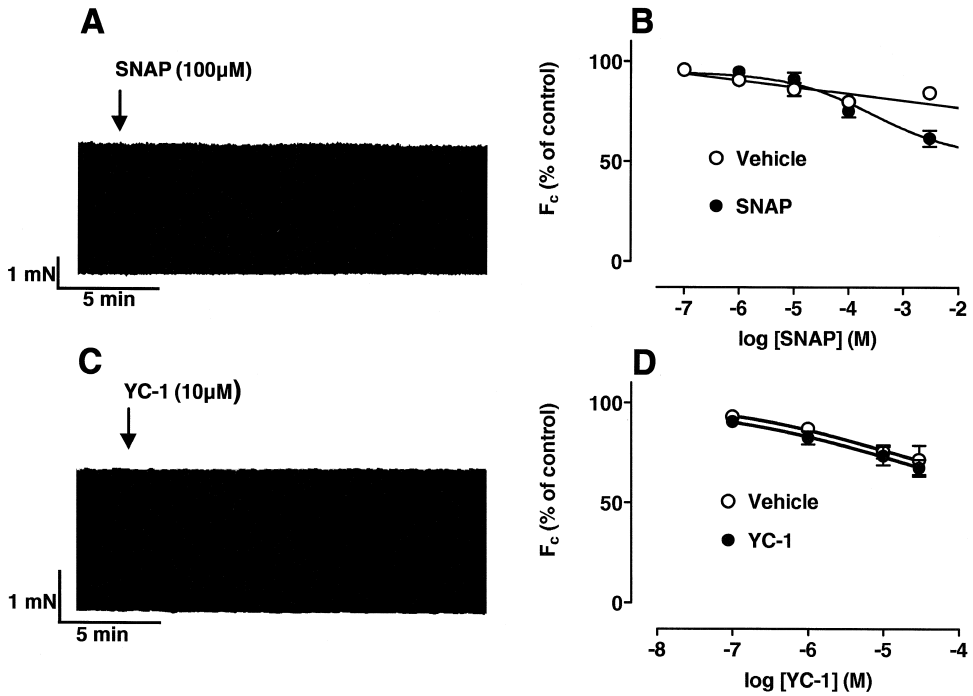

Fig. 1. Effects of *S*-nitroso-*N*-acetylpenicillamine (SNAP) and YC-1 on F_c in cardiac muscle from adult rats. (A, C) Time course of F_c. The arrows indicate the time of application of *S*-nitroso-*N*-acetylpenicillamine (100 μM, A) and YC-1 (10 μM, C), respectively. (B, D) Concentration-dependent effects of *S*-nitroso-*N*-acetylpenicillamine (B) and YC-1 (D) on F_c. Under control conditions, the corresponding amount of solvent (vehicle) was added without any drug. The data obtained with *S*-nitroso-*N*-acetylpenicillamine were fitted by a sigmoidal function with an EC_{50} of 0.28 mM. Mean values ± S.E.M. ($n = 6–9$).

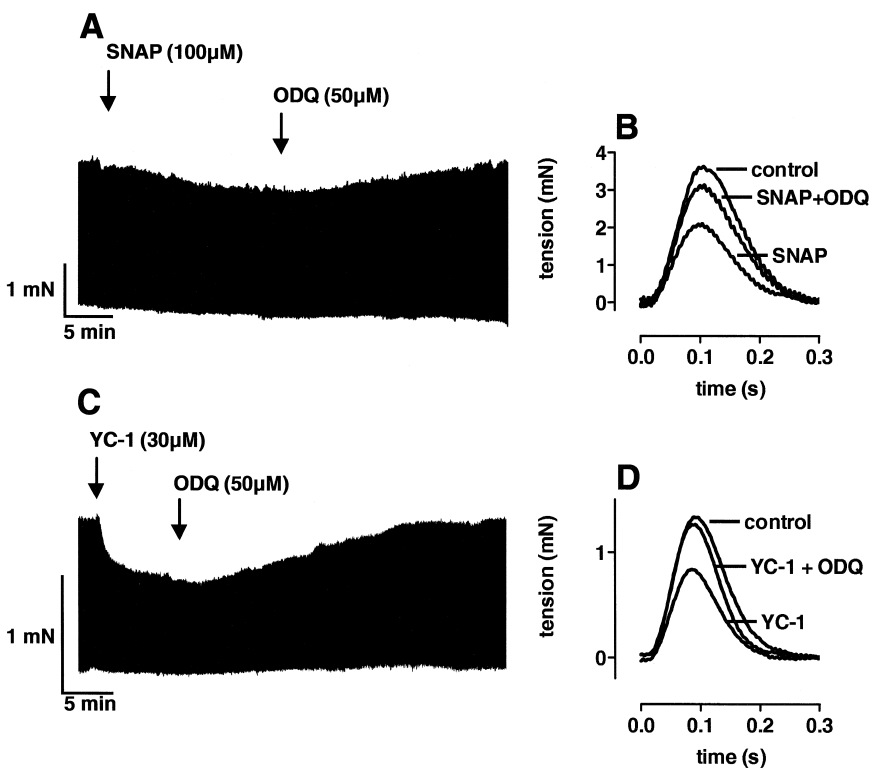

Fig. 2. Effects of *S*-nitroso-*N*-acetylpenicillamine (SNAP) and YC-1 on F_c in cardiac muscle from neonatal rats. (A, C) Time course of F_c. The arrows indicate the time of application of *S*-nitroso-*N*-acetylpenicillamine (100 μM, A), YC-1 (30 μM, C), or ODQ (50 μM, A and C), respectively. (B, D) Time course of single contractions. Single contractions are graphically superimposed under control conditions, in the presence of either *S*-nitroso-*N*-acetylpenicillamine (100 μM, B) or YC-1 (30 μM, D), and after addition of ODQ (50 μM).

rived from the blood, hearts from adult rats were perfused with Tyrode's solution via the aorta using the Langendorff set-up whereas hearts from neonatal rats were dissected and washed twice in Tyrode's solution.

2.5. Chemicals

Na_2GTP was obtained from Boehringer (Mannheim, Germany); ODQ and *S*-nitroso-*N*-acetylpenicillamine were from Calbiochem (Bad Soden, Germany). YC-1 was a gift from Hoechst (Germany). All other chemicals used were as pure as commercially available and purchased from Sigma (Deisenhofen, Germany). Stock solutions of YC-1 and ODQ were prepared in dimethylsulfoxide (DMSO) and further diluted to achieve the final bath concentration. The final amount of DMSO (vehicle) in test solutions was used in control experiments.

2.6. Evaluation of results

Data are presented as original recordings or expressed as means ± S.E.M. F_c was measured as the difference between resting and peak tension. Changes in aortic tension are expressed as percentage of phenylephrine-induced tension. The magnitude of $I_{Ca(L)}$ ($I_{Ca(L)peak}$) was measured as the difference between peak inward and steady-state current values at the end of the voltage pulse. Concentration–response curves were fitted by sigmoidal functions (correlation coefficient > 0.99) using GraphPad Prism 3.0 (GraphPad Software, San Diego, CA, USA). Statistical analysis was performed using either paired or unpaired Student's *t*-test and a two-way analysis of variance (repeated measurements design). *P*-values < 0.05 were considered as significant and are indicated by asterisks. Absence of significance is indicated by N.S.

3. Results

In heart muscle strips from adult rats, *S*-nitroso-*N*-acetylpenicillamine at 100 μM and YC-1 at 10 μM did not significantly influence the F_c (Fig. 1A and C) or the time course of single contractions (not shown). These concentrations have been shown to be fully effective in smooth muscle preparations (Henry et al., 1989; Wegener et al., 1997). A slight decrease of F_c was frequently observed at higher concentrations of the drugs and was related to the amount of solvent used (vehicle; Fig. 1B and D). The reduction of F_c was only statistically different from that under control conditions for *S*-nitroso-*N*-acetylpenicillamine at 3 mM ($P < 0.05$). A comparable high concentration of YC-1 was not tested since the drug was not soluble above 30 μM in aqueous solution without increasing the amount of the solvent DMSO above 1%.

In heart muscle strips from neonatal rats, both *S*-nitroso-*N*-acetylpenicillamine (100 μM) and YC-1 (30 μM) reduced F_c (Fig. 2). The time course of single contractions was not influenced by either drug (Fig. 2B and D). The reduction of F_c by *S*-nitroso-*N*-acetylpenicillamine (100 μM) or YC-1 (30 μM) was reversed by the application of ODQ (50 μM; Fig. 2A and C, respectively).

These effects of *S*-nitroso-*N*-acetylpenicillamine and YC-1 on F_c were dependent on the drug concentration; the EC_{50} values amounted to 20 μM with *S*-nitroso-*N*-acetylpenicillamine and to 1.2 μM with YC-1 (Fig. 3A and B). At the maximal concentrations tested in this preparation, *S*-nitroso-*N*-acetylpenicillamine (300 μM) and YC-1 (30 μM) reduced F_c to 52 ± 4% of control ($n = 9$) and to 58 ± 3% of control ($n = 12$), respectively, which resembles the effects of 100 μM 8-Br-cGMP on atrial F_c (Nawrath et al., 1995). In the presence of ODQ (50 μM), the effects of both drugs were significantly reduced (Fig. 3A and B; $P < 0.01$).

In ventricular cardiomyocytes from neonatal rats, *S*-nitroso-*N*-acetylpenicillamine (100 μM) and YC-1 (30 μM) reduced the magnitude of $I_{Ca(L)}$ by about 40% (Fig. 4). These effects were absent in ventricular cardiomyocytes from adult rat hearts (Fig. 5).

The effectiveness of the cGMP signalling pathway was investigated in cardiac and smooth muscle preparations

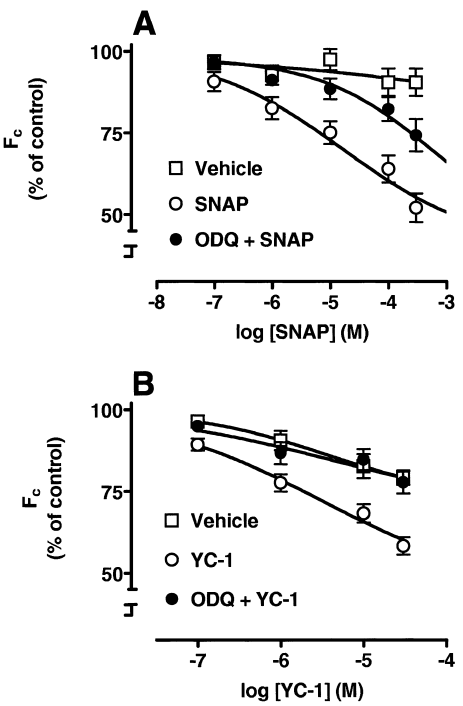

Fig. 3. Concentration-dependent effects of *S*-nitroso-*N*-acetylpenicillamine (SNAP, A) and YC-1 (B) on F_c in cardiac muscle from neonatal rats. The EC_{50} values were 20 μM and 1 μM, respectively. Under control conditions, the corresponding amount of solvent (vehicle) was added without any drug. In the presence of ODQ (50 μM), the effects of both substances were significantly reduced, as evaluated by a two-way analysis of variance (repeated measurements design; $P < 0.05$, respectively). Mean values ± S.E.M. ($n = 6–9$).

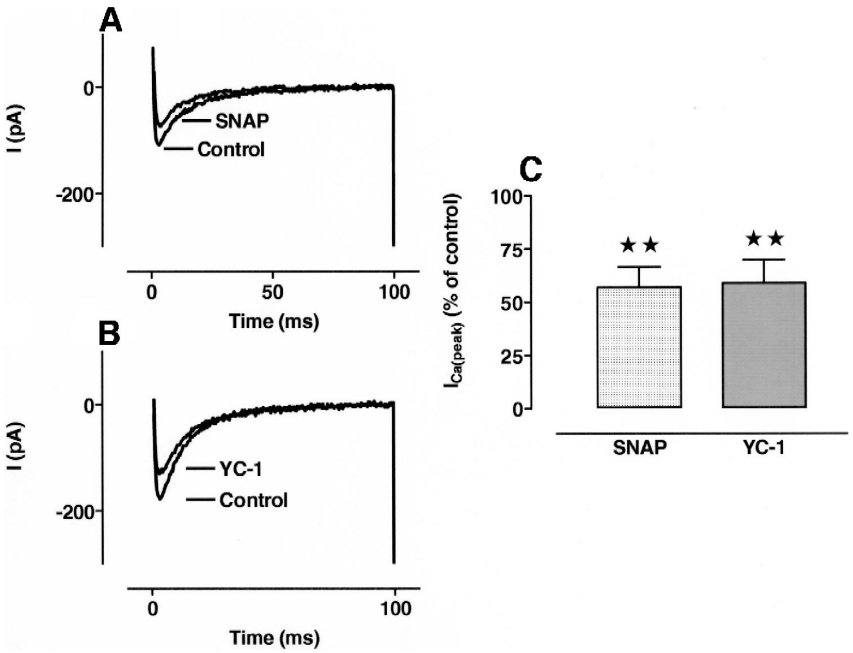

Fig. 4. Effects of *S*-nitroso-*N*-acetylpenicillamine (SNAP) and YC-1 on $I_{Ca(L)}$ in cardiomyocytes from neonatal rats. (A, B) Original recordings. Current traces under control conditions and after addition of *S*-nitroso-*N*-acetylpenicillamine (100 μM, A) or YC-1 (30 μM, B) are graphically superimposed. (C) Magnitude of $I_{Ca(L)}$ ($I_{Ca(peak)}$; in % of control). $I_{Ca(peak)}$ was 57 ± 10% in the presence of *S*-nitroso-*N*-acetylpenicillamine (100 μM and 59 ± 11% in the presence of YC-1 (30 μM). Mean values ± S.E.M. ($n = 3–4$).

from adult and neonatal animals to elucidate possible developmental differences in the expression of the responsive elements. In cardiac preparations, the effects of the membrane-permeable cGMP analogue, 8-Br-cGMP, on F_c were studied. 8-Br-cGMP (100 μM) reduced F_c to 51 ± 4% of control ($n = 9$) in preparations from neonatal ani-

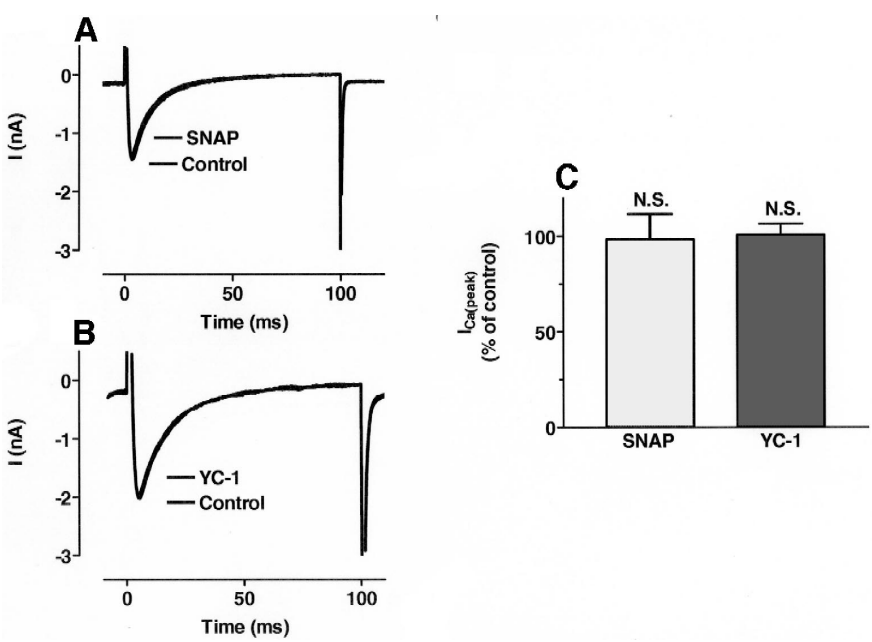

Fig. 5. Effects of *S*-nitroso-*N*-acetylpenicillamine (SNAP) and YC-1 on $I_{Ca(L)}$ in cardiomyocytes from adult rats. (A, B) Original recordings. Current traces under control conditions and after addition of *S*-nitroso-*N*-acetylpenicillamine (100 μM, A) or YC-1 (30 μM, B) are graphically superimposed. (C) Magnitude of $I_{Ca(L)}$ (in % of control). $I_{Ca(peak)}$ was 98 ± 13% in the presence of *S*-nitroso-*N*-acetylpenicillamine (100 μM) and 93 ± 6% in the presence of YC-1 (30 μM). Mean values ± S.E.M. ($n = 8–11$).

mals and to 64 ± 5% of control ($n = 4$) in preparations from adult animals. In aortic preparations, the relaxant effects of *S*-nitroso-*N*-acetylpenicillamine were investigated. *S*-nitroso-*N*-acetylpenicillamine relaxed pre-contracted aortic preparations from adult and neonatal animals with EC_{50} values of 44 and 27 nM, respectively.

The differential effects of *S*-nitroso-*N*-acetylpenicillamine and YC-1 in cardiac muscle from adult and neonatal animals may be related to differences in the content of myoglobin. The myoglobin content was, therefore, determined in both tissues. It was found that the hearts from adult rats contained about threefold more myoglobin than the hearts from neonatal rats (160 vs. 47 mg/100 g wet weight).

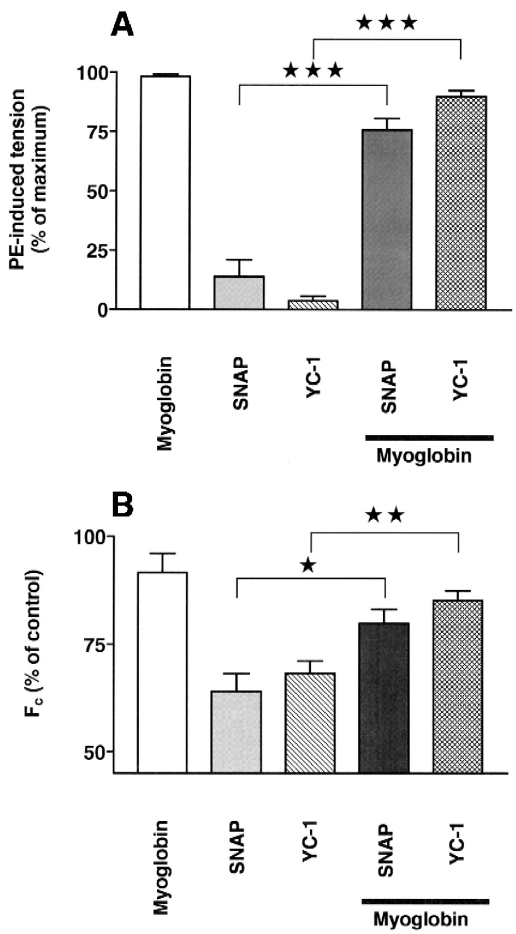

Fig. 6. Effects of YC-1 and *S*-nitroso-*N*-acetylpenicillamine (SNAP) on tension in aortic rings and on F_c in myocardial preparations from neonatal rats in the absence and presence of extracellularly applied myoglobin. (A) Effects of myoglobin on YC-1- and *S*-nitroso-*N*-acetylpenicillamine-induced relaxation of aortic rings pre-contracted by phenylephrine (3 μM). Myoglobin (300 μM) did not affect tension (98 ± 1% of control). In the presence of myoglobin (300 μM), *S*-nitroso-*N*-acetylpenicillamine (1 μM) reduced phenylephrine-induced tension (in % of control) to 76 ± 5 and YC-1 (3 μM) to 90 ± 2. Without any myoglobin, *S*-nitroso-*N*-acetylpenicillamine (1 μM) reduced phenylephrine-induced tension (in % of control) to 14 ± 7 and YC-1 (3 μM) to 4 ± 2. Mean values ± S.E.M. ($n = 3$–6). (B) Effects of myoglobin on the YC-1- and *S*-nitroso-*N*-acetylpenicillamine-induced decrease in cardiac F_c. Myoglobin (30 μM) reduced F_c to 92 ± 4% of control. In the presence of myoglobin (30 μM), *S*-nitroso-*N*-acetylpenicillamine (100 μM) and YC-1 (10 μM) reduced F_c (in % of control) to 80 ± 3 and to 85 ± 2, respectively. Without extracellularly applied myoglobin, *S*-nitroso-*N*-acetylpenicillamine (100 μM) and YC-1 (10 μM) reduced F_c (in % of control) to 64 ± 4 and to 68 ± 3, respectively. Mean values ± S.E.M. ($n = 4$–12).

Since myoglobin has been reported to act as a scavenger of NO molecules (Mittal et al., 1978), its possible interaction with YC-1 was investigated. For this purpose, the relaxant effects of *S*-nitroso-*N*-acetylpenicillamine and YC-1 on pre-contracted rat aortic rings were studied in the presence of extracellular myoglobin. At 300 μM myoglobin (5 mg/ml), the effects of both *S*-nitroso-*N*-acetylpenicillamine and YC-1 were significantly reduced (Fig. 6A). In contrast, a comparable amount of bovine serum albumin (5 mg/ml) did not prevent the relaxation induced by *S*-nitroso-*N*-acetylpenicillamine or YC-1 (not shown). The influence of extracellularly applied myoglobin on the effects of *S*-nitroso-*N*-acetylpenicillamine and YC-1 on F_c was also examined in myocardial preparations from neonatal rats. Myoglobin (30 μM) attenuated the effects of both *S*-nitroso-*N*-acetylpenicillamine and YC-1 (Fig. 6B).

4. Discussion

The present study has shown that the NO-donor *S*-nitroso-*N*-acetylpenicillamine as well as the activator of soluble guanylyl cyclase YC-1 decreased F_c and $I_{Ca(L)}$ more potently in the myocardium from neonatal rats than in the myocardium from adult rats. One reason for this difference may be a change in the expression of the NO/soluble guanylyl cyclase/cGMP signalling pathway during postnatal development. Both NO and YC-1 have been shown to activate soluble guanylyl cyclase (Murad, 1994; Friebe and Koesling, 1998). This enzyme may mediate their effects in the myocardium of neonatal rats since these effects were attenuated by the soluble guanylyl cyclase inhibitor ODQ. Interestingly, age-dependent changes in soluble guanylyl cyclase activity have been observed in some guinea pig smooth muscle tissues such as bladder and urethra (Wheeler et al., 1997) as well as in rat pulmonary tissue (Bloch et al., 1997). In aortic smooth muscle from neonatal and adult rats, there seems to be no difference in soluble guanylyl cyclase activity since the relaxant effects of *S*-nitroso-*N*-acetylpenicillamine were not statistically different in the two tissues. In rabbit ventricular myocardium, levels of cGMP were four to fivefold higher in neonatal tissue than in adult tissue (Kumar et al., 1994), which points to different levels of soluble guanylyl cyclase activity. In the present study, activation of soluble guanylyl cyclase by an NO donor or by YC-1 induced negative inotropic effects in the myocardium from neonatal, but not adult, rats although the membrane-permeable analogue of cGMP, 8-Br-cGMP, was effective in both tissues. In addition, it has recently been

reported that the expression of an NO-producing isoenzyme and cGMP levels are changed during development in the murine heart (Ji et al., 1999): embryonic myocardium (E9–E13-old) displayed a strong expression of the inducible NO synthase (iNOS) and a high cGMP content whereas no iNOS and a very low cGMP content were detected in E16-old myocardium. Taken together, these findings and the findings of the present study indicate that soluble guanylyl cyclase is important during early embryonic development of the rat myocardium but is of minor importance after maturation and suggest a switch in the signalling cascades after birth concerning the NO/soluble guanylyl cyclase/cGMP pathway. The situation may change again during ageing since a slightly higher expression of soluble guanylyl cyclase has recently been reported in 17-month-old rats as compared to 6-week-old rats (Ruetten et al., 1999).

The dominant mechanism involved in the different action of *S*-nitroso-*N*-acetylpenicillamine and YC-1 on neonatal and adult myocardium is thought to be the regulation of $I_{Ca(L)}$ by cGMP. It has been shown, using murine embryonic stem cells as an in vitro model of cardiomyogenesis, that cholinergic inhibition of basal $I_{Ca(L)}$ is mediated by the NO/cGMP system in early-developmental stage cardiomyocytes (Ji et al., 1999). In late developmental stage cardiomyocytes, this pathway does not influence basal but reduces β-adrenoceptor-stimulated $I_{Ca(L)}$, which has also been shown in adult mammalian cardiomyocytes by other investigators (i.e. Hartzell and Fischmeister, 1986; Levi et al., 1989). In contrast, a membrane-permeable analogue of cGMP (8-Br-cGMP) and the NO-donor nitrosoglutathion have been reported to increase basal $I_{Ca(L)}$ in neonatal but not adult rabbit cardiomyocytes (Kumar et al., 1997). Therefore, although differences in the NO/cGMP signalling pathway exist during postnatal development, the corresponding effects on $I_{Ca(L)}$ may depend on the species used and are probably determined by the expression of cGMP-dependent protein kinases and/or cGMP-dependent phosphodiesterases (Kumar et al., 1997).

Alternatively, the different action of the NO-donor and YC-1 on neonatal and adult myocardium, shown in the present study, may be explained by developmental changes in the content of myoglobin. Adult rat myocardium contained threefold more myoglobin than neonatal rat myocardium (Rakusan et al., 1965; this study), which could also be seen from the lighter colour of muscles from young as compared to adult animals. Myoglobin represents an endogenous scavenger of free NO molecules and, therefore, inhibits activation of soluble guanylyl cyclase by NO or NO-donors (Mittal et al., 1978; Martin et al., 1985). The different potency of the NO-donor *S*-nitroso-*N*-acetylpenicillamine to influence cardiac contractility or $I_{Ca(L)}$ in the myocardium from neonatal rats and adult rats may therefore be due to the high myoglobin content in the latter tissue. The lack of effects of NO-donors on myocardial cGMP levels in right atrium and papillary muscle from rabbits have also been ascribed to the high myoglobin content found in these tissues (Ishibashi et al., 1993). Since extracellular myoglobin at concentrations reported to be present in striated muscle (200–300 μM; Doeller and Wittenberg, 1991; Jurgens et al., 1994) abolished the effects of YC-1 on pre-contracted smooth muscle and, even at 30 μM, on F_c in myocardial preparations from neonatal rats (this study), the absence of effects of YC-1 on the adult myocardium (Wegener et al., 1997; this study) may also be due to its high myoglobin content. However, the precise mechanism by which myoglobin, but not bovine serum albumin, counteracts the effects of YC-1 still remains unclear. With respect to these findings, it is suggested that in striated muscles containing a high myoglobin level, NO-donors and YC-1 are not able to activate soluble guanylyl cyclase due to the scavenger function of intracellular myoglobin.

From this point of view, the discrepancy in the observed effects of NO and related substances on cardiac contractility in mammals (i.e. negative inotropic effects; Smith et al., 1991; Brady et al., 1992; Flesch et al., 1997), and the absence of effects (Weyrich et al., 1994; Nawrath et al., 1995) may be related to differences in the myoglobin concentration in the preparations and species used. For example, rat cardiac muscle contains sevenfold more myoglobin than guinea pig cardiac muscle (500 and 70 mg/100 g, respectively; O'Brien et al., 1992). The situation may be different in non-mammalian striated muscle preparations which contain lower myoglobin levels than mammalian striated muscle (O'Brien et al., 1992). Therefore, it seems possible that the described effects of NO-related substances and ODQ in frog cardiomyocytes (Méry et al., 1993; Abi-Gerges et al., 1997) are related to a low level of myoglobin in these cells. Since myoglobin has not been detected in smooth muscle preparations (Fasold et al., 1970; Ishibashi et al., 1993), *S*-nitroso-*N*-acetylpenicillamine and YC-1 are not prevented from activating soluble guanylyl cyclase in these tissues.

Evidence that NO is involved in a great number of pathological conditions is increasing, i.e. myocardial inflammation and septic cardiomyopathy. An enhanced expression of NO synthase isoforms and increased cGMP levels has been found in septic human hearts (Thoenes et al., 1996). In addition, the NO synthase-inhibitor N^G-monomethyl-L-arginine (L-NMMA) has been shown to inhibit cytokine-impaired contractility in isolated hamster papillary muscle (Finkel et al., 1992). Treatment with endotoxin reduced cardiac contractility in guinea pigs (Brady et al., 1992) and rats (Balligand et al., 1993), both effects being antagonised by L-NMMA. It is tempting to speculate that, in cardiomyopathy or cardiac hypertrophy, cardiomyocytes undergo changes in protein expression reminiscent to those in early developmental stages, i.e. enhancing the importance of the NO/soluble guanylyl cyclase signalling pathway and/or changing the content of myoglobin.

In summary, the present study suggests that the differences in action of *S*-nitroso-*N*-acetylpenicillamine and YC-1 in heart muscle of neonatal and adult rats are due to a postnatal change in the myoglobin content and/or in the expression of the NO/soluble guanylyl cyclase/cGMP signalling pathway.

Acknowledgements

This study was supported by grants from the Umweltministerium of Rheinland-Pfalz (Germany) to H.N. This work contains parts of the MD thesis of S.D.V.

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