Direct vasoconstrictor action of homologous angiotensin II on isolated arterial ring preparations in an elasmobranch fish

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Abstract

Arterial rings were prepared from the branchial artery, coeliac artery and ventral aorta of the Japanese dogfish *Triakis scyllia* and used to determine arterial contraction in a myograph. Noradrenaline caused a dose-dependent contraction (10^{-9}–3×10^{-6} M) that was completely inhibited by pre-treatment with 10^{-7} M phentolamine. Homologous dogfish angiotensin II (ANG II) ([Asn^1, Pro^3,Ile^5]-ANG II) also caused dose-dependent contraction (10^{-9}–3×10^{-6} M), but phentolamine had no effect on this response. Administration of dogfish angiotensin I (ANG-I) ([Asn^1,Pro^3,Ile^5,Gln^9]-ANG I) resulted in a contraction similar to that produced by ANG II and the effect could be blocked with 10^{-7} M captopril. The mammalian ANG II receptor antagonists [Sar^1,Ile^8]-ANG II and [Sar^1,Ala^8]-ANG II caused dose-dependent contractions of coeliac artery rings, but were less potent than homologous ANG I and ANG II. These results show that the contractile effect of [Asn^1,Pro^3,Ile^5]-ANG II is not mediated by the α-adrenergic system and contractions of arterial rings by noradrenaline and elasmobranch ANG II are mediated by separate vascular receptors. The elasmobranch ANG II vascular receptor may have co-evolved with the unusual structure of this peptide.

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Introduction

The renin–angiotensin system (RAS) has been identified in the majority of vertebrate groups studied so far, including elasmobranch fish (Takei *et al.* 1993). To date, the mammalian RAS has been the most extensively studied, but components of the system have been identified in all other vertebrate groups. The main biologically active element of the system appears to be the octapeptide angiotensin II (ANG II), which is formed by the cleavage of amino acids 9 and 10 from the decapeptide angiotensin I (ANG I) by ANG I-converting enzyme (ACE). In mammals, ANG II plays a major physiological role in the control of the cardiovascular system and in the maintenance of salt and water balance. ANG II is cleaved to angiotensin III (ANG III), which also has bioactivity, and angiotensin IV, the physiological function of which remains to be established (Kobayashi & Takei 1996).

The basic angiotensin structure is conserved throughout the vertebrate groups with interchange of asparagine and aspartic acid at position 1 and valine and isoleucine at position 5 in teleost and mammalian species respectively. The elasmobranch angiotensins are somewhat unusual and possess asparagine at position 1 and isoleucine at position 5, with a unique proline substitution at position 3 (Takei *et al.* 1993). Prior to the recent isolation of homologous dogfish angiotensin, elasmobranchs were widely believed to lack a RAS (Nishimura *et al.* 1970), so little information is available regarding the physiological role of ANG II in this vertebrate group.

In most other vertebrates, administration of heterologous and homologous ANG II produces a vasopressor response, and this has also been demonstrated in the dogfishes *Scyliorhinus canicula* and *Triakis scyllia* (Hazon *et al.* 1989, 1995). In lower vertebrates, catecholamines may play an important role in mediating the ANG II-induced pressor response. Using phentolamine as an α-adrenergic receptor blocker, partial inhibition of the ANG II pressor response was observed in the eel (Nishimura *et al.* 1978) and in the frog, turtle and chicken (Carroll & Opdyke 1982). Phentolamine completely abolished the [Asp^1,Val^3,Val^5]-ANG II-mediated pressor response in the spiny dogfish, *Squalus acanthias*, (Opdyke & Holcombe 1976), and injection of [Asn^1,Val^3,Val^5]-ANG II increased plasma concentrations of both adrenaline and noradrenaline (Opdyke *et al.* 1981). This evidence appears to indicate a role for catecholamines as the mediator of the ANG II pressor response in elasmobranchs.

However, due to the relatively recent discovery of elasmobranch ANG II, the majority of studies investigating the role of ANG II have used heterologous peptides. Considering the unique amino acid sequence of dogfish

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AII and the implications of this for the characteristics of dogfish ANG II receptors (Tierney *et al.* 1997*a*), it is important to utilise homologous ANG II for all further studies. The current study, therefore, investigates the presence of functional vascular ANG II receptors in a variety of arteries isolated from the Japanese dogfish, *Triakis scyllia*, and utilises homologous ANG I and ANG II. The interaction between catecholamines and ANG II in contracting these blood vessels is also assessed, particularly considering the recent report that phentolamine did not block the pressor effect of homologous [Asn^1,Pro^3,Ile^5]-ANG II *in vivo* (Tierney *et al.* 1997*b*).

Materials and Methods

Dogfish (*Triakis scyllia*) weighing approximately 1 kg, were caught in Sagami Bay, and purchased from Jougashima Fisheries Association, Kanagawa, Japan. They were maintained in a flow-through seawater aquarium at 20 °C for at least one week prior to use. Some fish were also kindly donated by Yokohama Hakkeijima Sea-Paradise, Yokohama, Japan.

Dogfish were killed by a blow to the head and pithed. The ventral aorta, first branchial artery and coeliac artery were immediately isolated and placed in ice-cold dogfish Ringer solution (240 mM NaCl, 7 mM KCl, 10 mM $CaCl_2$, 4·9 mM $MgCl_2.6H_2O$, 2·3 mM $NaHCO_3$, 0·5 mM $Na_2HPO_4.2H_2O$, 0·5 mM Na_2SO_4, 360 mM urea, 60 mM trimethylamine oxide, 0·1% glucose, pH 7·8 oxygenated with 95% O_2–5% CO_2). Each arterial ring was cut so as to be 3–4 mm wide, set between the wires of a double myograph (Kishimoto Ikasangyo, Tokyo, Japan) and placed in a trough containing 5 ml Ringer solution, and continuously aerated with 95% O_2–5% CO_2 at 20 °C. The tension was gradually increased on the arterial ring until the contraction caused by 25 mM KCl was detectable (approximately 5 mN). The vessels were then left for 1 h to establish a stable baseline. The contractile action of [Asn^1,Pro^3,Ile^5]-ANG II, and dogfish [Asn^1,Pro^3,Ile^5,Gln^9]-ANG I (Peptide Institute Inc., Osaka, Japan) or noradrenaline (Research Biochemicals Inc., Tokyo, Japan) was examined by successive addition of increasing concentrations of the test substances (cumulative concentration–response experiments). The effects of the α-adrenoreceptor blocker phentolamine (CIBA-GEIGY Japan Ltd, Takarazuka, Japan), competitive ANG II receptor antagonists, [Sar^1,Ile^8]-ANG II or [Sar^1,Ala^8]-ANG II, (Peptide Institute Inc.) or the ANG I-converting enzyme inhibitor, captopril (Sankyo Pharmaceutics Co., Ltd, Tokyo, Japan), were examined by adding 10^{-7} M of the substance 10 min before application of ANG II, ANG I or noradrenaline. The effects of the ANG II receptor antagonists, dogfish ANG I and captopril were investigated in the coeliac arterial ring preparation alone. A period of at least 1 h was allowed between successive applications.

In all experiments, each experimental group consisted of at least six preparations, except for the branchial artery with noradrenaline and phentolamine and the coeliac artery with dogfish ANG I, which had four preparations. All values are presented as means ± S.E.M. Changes in contraction of arterial rings compared with baseline values were analysed by analysis of variance (ANOVA) followed by Dunnett's test. To compare the mean value of a specific experimental group with and without the appropriate blocker, Student's *t*-test was used.

Results

Arterial ring preparations from all three blood vessels showed a concentration-dependent response to noradrenaline (Fig. 1). Contraction was significantly greater than baseline values at concentrations of 3×10^{-7} M noradrenaline for the branchial artery, 10^{-7} M for the ventral aorta and 10^{-6} M for the coeliac artery ($P<0·01$). The pre-treatment of vessels with 10^{-7} M phentolamine completely abolished the vasoconstrictor effect of noradrenaline (Fig. 1) and for all three vessels, contraction did not increase significantly from basal values at all concentrations of noradrenaline used. Phentolamine caused significant inhibition of noradrenaline-induced contraction at noradrenaline concentrations of 3×10^{-6} M to 3×10^{-7} M in the branchial and coeliac artery and 3×10^{-6} M to 3×10^{-8} M in the ventral aorta ($P<0·05$ Student's *t*-test).

The addition of [Asn^1,Pro^3,Ile^5]-ANG II also caused a concentration-dependent contraction which was highly significant compared with baseline values (Fig. 2). Contraction was significantly increased at ANG II concentrations of 3×10^{-7} M for the branchial artery and ventral aorta and 10^{-7} M for the coeliac artery ($P<0·01$). In these experiments, pre-treatment with phentolamine had no effect on the contraction caused by ANG II (Fig. 2). As the coeliac artery showed significant increases in contraction at a lower concentration of ANG II than the other vessels and as the response to each individual dose of ANG II was less variable, further investigations were conducted only on this vessel.

The contraction caused by dogfish ANG II was examined further by using [Sar^1,Ile^8]-ANG II and [Sar^1,Ala^8]-ANG II which, at least in mammals, are regarded as angiotensin II receptor antagonists. Figure 3 shows that in the coeliac artery, both peptides caused a significant dose-dependent contraction. However, these effects were at concentrations significantly higher ($P<0·01$) than dogfish ANG II, 10^{-6} M for [Sar^1,Ile^8]-ANG II, and 10^{-4} M for [Sar^1,Ala^8]-ANG II, compared with 10^{-7} M for dogfish ANG II. Dogfish [Asn^1,Pro^3,Ile^5,Gln^9]-ANG I also caused a significant dose-dependent contraction (Fig. 4) which was at least as potent as that of [Asn^1,Pro^3,Ile^5]-AII. The effects of dogfish ANG I, but not dogfish ANG II, were completely abolished after pre-treatment with the converting enzyme inhibitor captopril (Fig. 4).

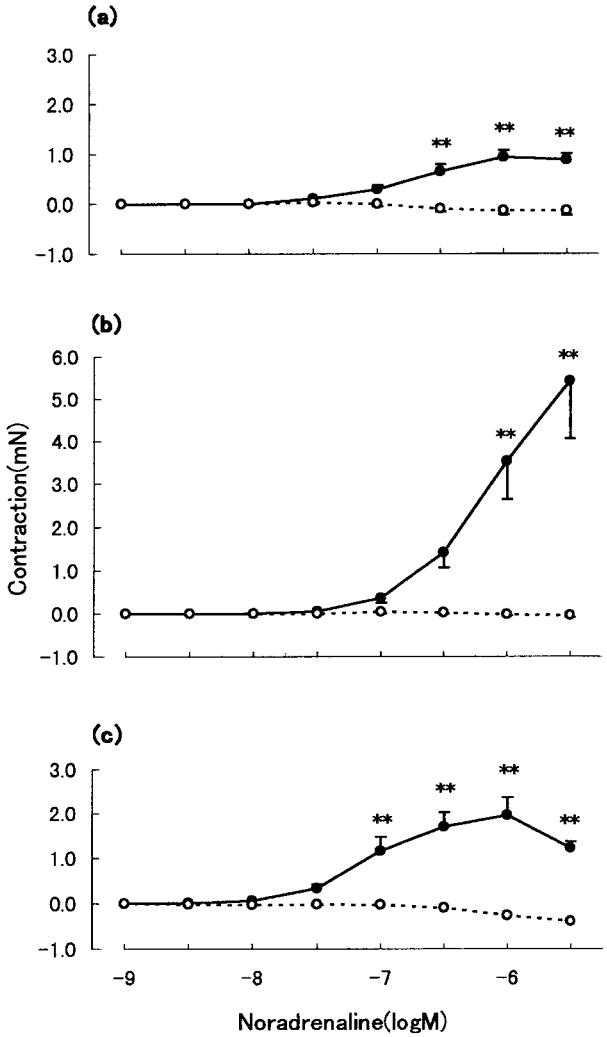

Figure 1 Contraction of (a) branchial, (b) coeliac, and (c) ventral aorta arterial rings in response to noradrenaline without (●) and in the presence of 10^{-7} M phentolamine (○). Data are expressed as means ± S.E.M. $^{**}P<0{\cdot}01$ compared with baseline values (ANOVA and Dunnett's test).

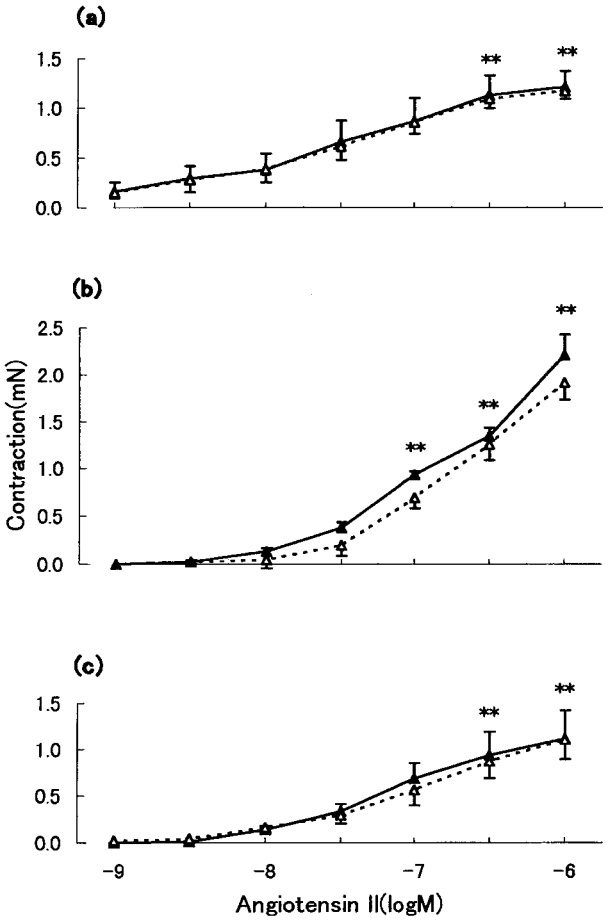

Figure 2 Contraction of (a) branchial, (b) coeliac, and (c) ventral aorta arterial rings in response to angiotensin II without (▲) and in the presence of 10^{-7} M phentolamine (△). Data are expressed as means ± S.E.M. $^{**}P<0{\cdot}01$ compared with baseline values (ANOVA and Dunnett's test).

Discussion

The contraction of blood vessels caused by the vasoconstrictor effect of the catecholamine, noradrenaline, demonstrated *in vitro* in this study, is in agreement with the results obtained in *Squalus acanthias* (Carroll 1981). The blockade of noradrenaline-mediated vasoconstriction with phentolamine indicates the importance of α-adrenergic receptors in this response. Utilising homologous [Asn^1,Pro^3,Ile^5]-ANG II, a contraction of arterial rings prepared from all three vessels was obtained. This is in contrast to the results of a previous study (Carroll 1981) that was unable to demonstrate any contraction in coeliac artery preparations or changes in mesenteric microcirculatory blood flow utilising [Asn^1,Val^3,Val^5]-ANG II. The unusual amino acid sequence of the dogfish ANG II molecule means that many previous studies utilising heterologous peptides may have produced misleading results. For example, Takei *et al.* (1993) showed that homologous dogfish ANG I was more potently vasopressor (by a factor of 22·6) than rat ANG I in the dogfish *in vivo*, with the reverse relationship occurring in the rat.

The vasoconstriction induced by dogfish ANG II was not blocked by the addition of phentolamine, a result which contrasts sharply with other studies. A number of reports have pointed to an important role for catecholamines in mediating the ANG II vascular response in dogfish; phentolamine blocked the *in vivo* vasopressor response to [Asn^1,Val^3,Val^5]-ANG II (Opdyke & Holcombe 1976) and the concentration of plasma adrenaline and noradrenaline increased within one minute after injection of [Asn^1,Val^3,Val^5]-ANG II, leading to the

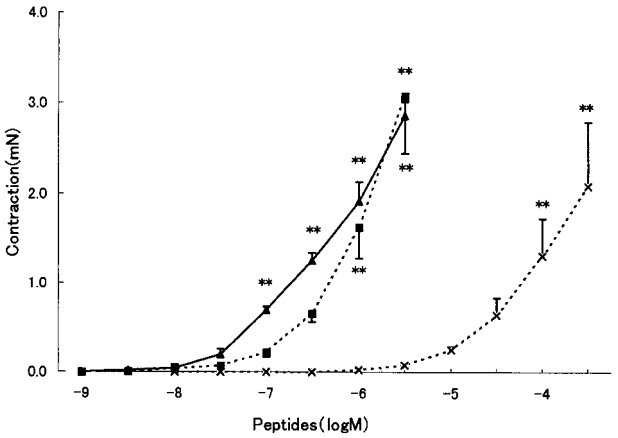

Figure 3 Contraction of coeliac artery rings in response to cumulative concentrations of angiotensin II (▲), [Sar^1,Ile^8]-angiotensin II (■) and [Sar^1,Ala^8]-angiotensin II (■). Data are expressed as means ± S.E.M. $^{**}P<0{\cdot}01$ compared with baseline values (ANOVA and Dunnett's test).

hypothesis that the ANG II effects were mediated solely by catecholamines or that noradrenaline and ANG II were acting via a common receptor (Opdyke & Holcombe 1976). However, the lack of phentolamine inhibition of dogfish ANG II-mediated contraction in the present studies suggests that the effects of noradrenaline and ANG II are mediated by separate receptors. The difference in results could reflect different ANG II structures in these two species or a species-dependent response to [Asn^1,Val^3,Val^5]-ANG II. Phylogenetically, *Squalus acanthias* (Squaliformes) and *Triakis scyllia* (Lamniformes) represent different orders of the elasmobranchii and certainly in terms of the structure of C-type natriuretic peptide, there are four amino acid substitutions between the two species (Takano *et al.*, 1994). Alternatively, it may be that

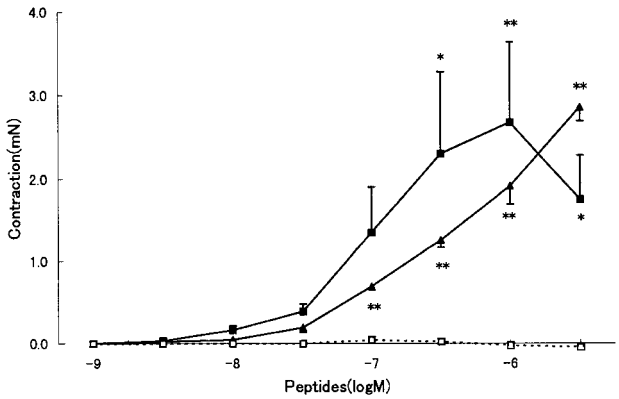

Figure 4 Contraction of coeliac artery rings in response to cumulative concentrations of angiotensin I (■), angiotensin II (▲) and angiotensin I with captopril (□). Data are expressed as means ± S.E.M. $^{**}P<0{\cdot}01$ compared with baseline values (ANOVA and Dunnett's test).

the vascular and chromaffin tissue ANG II receptors are different, with a highly specific vascular receptor which recognises only homologous dogfish ANG II and a less specific receptor type in the chromaffin tissue which binds both [Asn^1,Pro^3,Ile^5]-ANG II and [Asn^1,Val^3,Val^5]-ANG II and subsequently leads to catecholamine release.

The unusual amino acid sequence of the dogfish ANG II molecule means that the elasmobranch ANG II receptor may have co-evolved with the peptide and the effects of [Sar^1,Ile^8]-ANG II and [Sar^1,Ala^8]-ANG II, which are receptor antagonists in mammals, lend support to this possibility. Both peptides actually caused contraction in coeliac artery ring preparations. This is in agreement with recent *in vivo* studies in which these peptides also caused dose-dependent increases in mean arterial blood pressure and did not inhibit the pressor response to dogfish ANG II (Hazon *et al.* 1995). The variable effect of these mammalian receptor antagonists has been shown in other vertebrate groups (for review see Kobayashi & Takei 1996). For example [Sar^1,Ile^8]-ANG II and [Sar^1,Thr^8]-ANG II had only weak antagonistic activities in the eel and higher doses displayed agonistic effects (Nishimura *et al.* 1978). These studies also emphasise the importance of ACE in the physiological action of RAS. Captopril has previously been shown to block completely the *in vivo* blood pressure response to ANG I (Hazon *et al.* 1995) and in these studies captopril also blocked the effect of dogfish ANG I, but not dogfish ANG II, in contracting isolated arterial ring preparations. This suggests that the isolated arterial tissue possesses ACE activity and it may be that this is of endothelial origin. In teleost fish, captopril appears to be an effective inhibitor of ACE (Carrick & Balment 1983, Kenyon *et al.* 1985, Tierney *et al.* 1995), whereas in other vertebrates, depending on the group, captopril may show complete or only partial blockade of the conversion of ANG I to ANG II (for review see Kobayashi & Takei 1996).

The present study has demonstrated that homologous [Asn^1,Pro^3,Ile^5]-ANG II causes a dose-dependent contraction of isolated arterial rings prepared from the branchial and coeliac artery and ventral aorta of dogfish. This effect is not blocked by pre-treatment with the α-adrenergic blocker, phentolamine and this suggests that the vasoconstrictor effects of noradrenaline and ANG II are mediated through separate receptors. The lack of inhibition of the ANG II response by mammalian ANG II receptor antagonists lends support to the theory that the unusual elasmobranch ANG II peptide structure and its receptor have co-evolved.

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