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Competitive inhibition of Asp[1]-β-amide-Val[5]-angiotensin II by Sar[1]-Ile[5]-Ile[8]-angiotensin II in cat isolated cardiac muscle and coronary vessels*

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Interaction of Asp[1]-β-amide-Val[5]-angiotensin II with the 8-substituted analogue of Asp[1]-Ile[5]-angiotensin II, Sar[1]-Ile[5]-Ile[8]-angiotensin II, has been examined on the isolated perfused heart and isolated papillary muscle of the cat. The constrictor effect of angiotensin in the coronary vessels as well as its positive inotropic effect in the heart muscle have been shown to be competitively inhibited by Sar[1]-Ile[5]-Ile[8]-angiotensin II. The antagonistic potency and the duration of the antagonistic effect of the analogue in both coronary vessels and myocardium have been evaluated separately. The analogue has a higher antagonistic potency in coronary vessels than in heart muscle. The duration of the antagonistic effect of Sar[1]-Ile[5]-Ile[8]-angiotensin II was found to be longer in the heart muscle than in coronary vesesels. The possible mechanism of the antagonistic effect of Sar[1]-Ile[5]-Ile[8]-angiotensin II against Asp[1]-β-amide-Val[5]-angiotensin II is discussed.

Angiotensin has a dose-dependent positive inotropic action on mammalian ventricular myocardium (Beaulnes, 1963; Fowler & Holmes, 1964; Koch-Weser, 1964). However, the mechanism of the inotropic action of angiotensin has not been well established. It has been suggested that the greater part of this effect on heart muscle is due to release of catecholamines from adrenergic neurons (Beaulnes, 1963). Other investigators, however, have observed that the inotropic effect of the octapeptide did not depend upon myocardial catecholamine content (Fowler & Holmes, 1964; Koch-Weser, 1965).

Recently, several 8-substituted analogues of Asp[1]-Ile[5]-angiotensin II and Asp[1]-Val[5]-angiotensin II, which competitively block myotropic, pressor, as well as the ganglion stimulating, action of the parent peptide, have been reported (Türker, Page & Bumpus, 1974).

The interaction of Asp[1]-β-amide-Val[5]-angiotensin II (A II) and 8-substituted analogues has not been studied in cardiac muscle. The data presented here indicate that Sar[1]-Ile[5]-Ile[8]-angiotensin II (SIA II) (Türker, Hall & others, 1972) can competitively block the positive inotropic effect of A II in heart muscle as well as the vasoconstrictor effect of the octapeptide on the coronary vasculature.

MATERIALS AND METHODS

The experiments were carried out using isolated Langendorff perfused heart preparations and isolated papillary muscle from 25 mongrel cats of either sex, 2 to

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3·5 kg, anaesthetized with ether. The heart was perfused (through a cannula inserted into the ascending aorta) with Krebs solution (mM litre^{-1}) NaCl 112, $NaHCO_3$ 25, KCl 5, NaH_2PO_4 1, $MgCl_2$ 0·5, $CaCl_2$ 2·5 and dextrose 11·5 at 35° equilibrated with 5% CO_2 and oxygen. Other branches of the aorta were tied off so that all of the perfusion fluid was forced through the coronary arteries and the return was discarded. Krebs' solution was perfused by a multispeed peristaltic pump at a rate from 14·5 to 17·5 ml min^{-1}. This induced an initial pressure of 15 to 20 mm Hg and gradually increased to 30 to 40 mm Hg within 30 min and was kept constant throughout the experiments. Perfusion pressure of the coronary arteries was recorded by a Statham pressure transducer (Model P 23 Dc). A force-displacement transducer (Grass FT. 03) was attached to the apex of the heart and the contractility of the heart and coronary perfusion pressure was recorded on a Grass polygraph (Model 79 C). After an initial equilibration period the heart was perfused with Krebs solution for 30 min. Injections were made into the perfusion circuit proximal to the cannulated part of the aorta and the injected drugs were kept in a volume of 0·05 to 0·1 ml. SIA II was dissolved in Krebs solution and was continuously infused at a rate of 0·025 ml min^{-1} with a multispeed slow injection apparatus (Palmer). This infusion did not cause any significant changes in coronary perfusion pressure when Krebs solution alone was infused.

In five cats, the hearts were removed and placed in aerated Krebs solution at room temperature (20°) immediately after ether anaesthesia. Two papillary muscles of each heart from the right ventricle were used. The mural end of each muscle was fixed to a muscle holder in a 50 ml isolated organ bath containing aerated Krebs solution at 37° and the tendinous end was tied with a silk thread to a force-displacement transducer (Grass FT. 03). A 0·5 g resting tension was applied to the muscle which was then stimulated through two punctate platinum electrodes, with square-wave pulses of 5 ms duration at a voltage of 50% above threshold and a frequency of 10 Hz min^{-1} to a Grass stimulator (Model S 8c).

The experiments were performed in the following order:

In 3 perfused hearts, the effect of SIA II was studied alone. Different doses of the analogue were infused continuously or given by single injections and its effect on coronary perfusion pressure and heart contractility was determined.

In another series of experiments the log dose-response curves of A II were determined in both parameters before and after addition of SIA II in the perfusing medium and isolated organ bath. The doses of A II were limited between 10^{-9} to 10^{-7} M in the whole perfused heart, since a rapid tachyphylaxis was observed in the preliminary experiments when the higher doses of the octapeptide (above of 10^{-7} M) were used. The pA_2 value of SIA II was determined in both preparations in order to compare the antagonistic potency of the analogue against A II in coronary vessels and myocardium (Schild, 1947). Noradrenaline, vasopressin, bradykinin, histamine, prostaglandin E_1, E_2, $F_{2\alpha}$ and acetylcholine were also used in both preparations to determine the specificity of the blocking effects of SIA II against A II. Statistical evaluation of the results was calculated using Student's *t*-test.

The following drugs were used: Asp^1-β-amide-Arg-Val-Tyr-Val^5-His-Pro-Phe^8 = Asp^1-β-amide-Val^5-angiotensin II (Hypertensin, Ciba AG, Basel), (−)-noradrenaline bitartrate (Farbwerke Hoechst AG, Frankfurt), Sar^1-Arg-Val-Tyr-Ile^5-His-Pro-Ile^8 = Sar^1-Ile^5-Ile^9-angiotensin II. The latter peptide has been synthesized in the Cleveland Clinic, U.S.A. (Khosla, Leese & others, 1972).

RESULTS

Concentrations of SIA II less than 10^{-7} M, did not decrease coronary perfusion pressure or contract the heart muscle. Higher doses, above 10^{-7} M, however, increased the coronary perfusion pressure and also produced a slight positive inotropic effect.

Coronary artery injections of A II caused a dose-dependent increase in perfusion pressure and elicited a positive inotropic effect (Fig. 1a). No tachyphylaxis was observed with either effect when A II was injected repeatedly at 8 to 10 min intervals. Both pressor and positive inotropic effects of A II were found to be inhibited when SIA II was added to the perfusing medium (Fig. 1b). The log dose-response curves of A II in both coronary perfusion pressure and heart muscle are straight lines at concentrations between 1·7 and 6·8 $\times$ 10^{-9} M. These curves are shifted to the right and parallel to the control in the presence of SIA II at two different concentrations

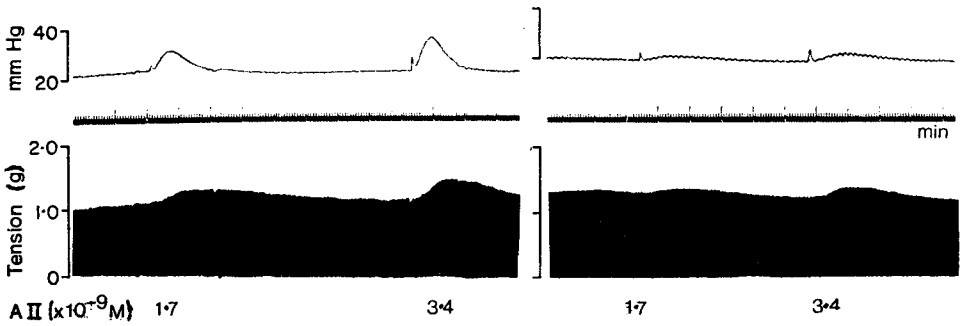

FIG. 1. Effect of A II on the coronary perfusion pressure and myocardial contractility of isolated perfused heart. (a) Control responses to 1·7 and 3·4 $\times$ 10^{-9}M concentration of A II; (b) Response to A II in the presence of SIA II (1·9 $\times$ 10^{-9}M). The upper tracing records the coronary perfusion pressure and the lower tracing demonstrates myocardial contractions.

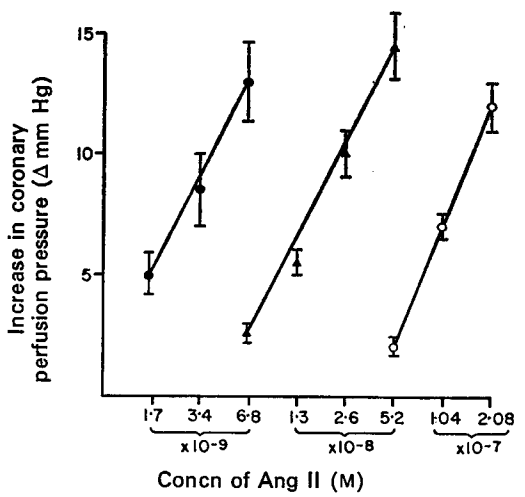

FIG. 2. Effect of A II on coronary perfusion pressure and its antagonism by SIA II. The first curve (●) represents the control response. The next two curves show the response of A II in the presence of SIA II (4·9 $\times$ 10^{-10}M) (▲) and (1·9 $\times$ 10^{-9}M) (○) respectively.

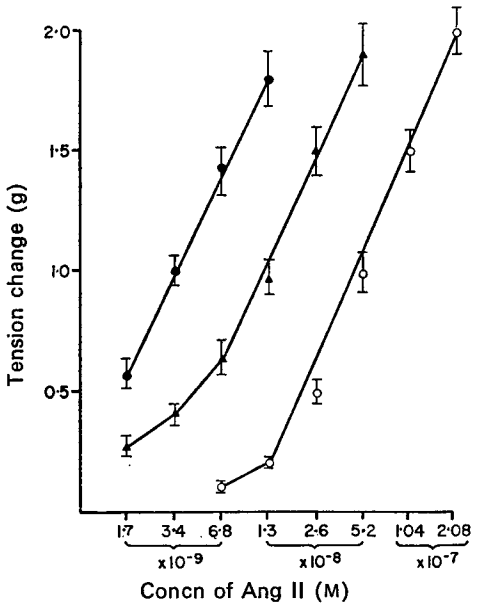

FIG. 3. Positive inotropic effect of A II and antagonism by SIA II on isolated perfused cat heart. The first curve (●) is the control response to varying doses of A II. The following two curves are the responses to A II in the presence of SIA II ($4{\cdot}9 \times 10^{-10}$M) (▲) and ($1{\cdot}4 \times 10^{-9}$M) (○) respectively.

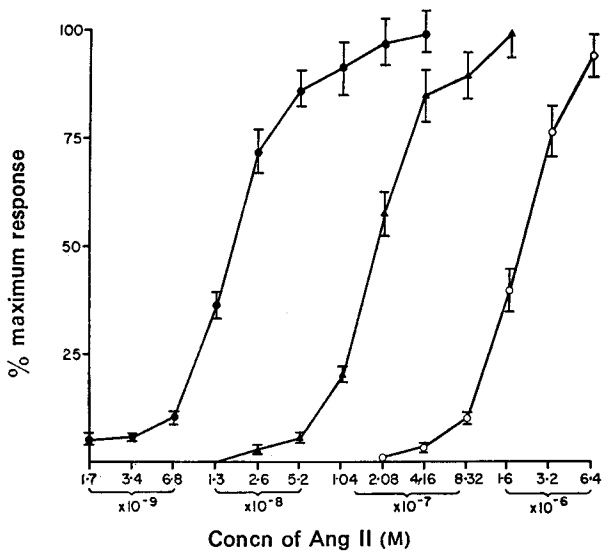

FIG. 4. Effect of A II on papillary muscle and its antagonism by SIA II. The control response to A II is represented by the first curve (●). The two subsequent curves show the response of A II in the presence of SIA II ($9{\cdot}9 \times 10^{-10}$M) (▲) and ($1{\cdot}9 \times 10^{-9}$M) (○) respectively.

(Figs 2 and 3). A II also induced a dose-dependent positive inotropic effect in isolated papillary muscle. This effect was inhibited competitively by lower concentrations of the analogue. The calculated log dose-response curve of A II with and without SIA II is shown in Fig. 4. Again the log dose-response curve of A II is found to be

shifted to the right and parallel to control. The pA_2 values of SIA II against A II in both perfused heart and isolated papillary muscle are calculated and the results are: pA_2 values (mean $\pm$ s.e. (n)) coronary vessels 9·24 $\pm$ 0·05 (7) (a); total heart muscle 8·46 $\pm$ 0·13 (7) (b); papillary muscle 8·57 $\pm$ 0·05 (7) (c); (a, b) $P < 0{\cdot}001$, (a, c) $P < 0{\cdot}001$, (b, c) $P > 0{\cdot}25$.

Single injections of SIA II through the coronary artery also antagonized the effects of injections of A II on both parameters. Complete recovery of the constrictor effect to A II in the coronary vessels was found to take 3 h while complete recovery of the positive inotropic effect of A II in the heart muscle took more than 4 h following single injections of SIA II. Under similar conditions, the complete recovery of the responses to A II occurred after 1 h in the coronary vessels and 1·5 h in the heart muscle following single injection of $3{\cdot}61 \times 10^{-9}$ M of Asp^1-Ile^5-Ile^8- A II (AIA II) which is another 8- substituted analogue of angiotensin (Yamamoto, Türker & others, 1972).

SIA II, at higher concentrations (above 10^{-7} M), did not inhibit the effects of noradrenaline on isolated perfused heart and isolated papillary muscle. Phentolamine ($1{\cdot}5 \times 10^{-5}$ M) and propranolol (5×10^{-6} M) did not block the effects of A II on coronary vessels and heart muscle, but completely antagonized the effect of noradrenaline. SIA II at the used and higher concentrations (above 10^{-7} M), did not inhibit the effects of vasopressin (10 μU), bradykinin (100 ng), histamine (1 μg), PGE_1, PGE_2, $PGF_{2\alpha}$ (100 ng) and acetylcholine (1 μg) on the isolated perfused heart.

DISCUSSION

SIA II is a long-lasting competitive inhibitor of A II (Türker & others, 1972). This antagonistic effect has been observed in *in vivo* experiments in cats and dogs as well as on rabbit isolated aorta. The present results indicate that this analogue can also inhibit the action of A II in coronary vessels and isolated heart muscle. This antagonism observed with SIA II appears to be competitive since log dose-response curves are shifted to the right and are parallel to control at different molar concentrations of the analogue. Even at 10^{-7} to 10^{-6} M concentrations of the analogue the maximum response to A II was still obtained, indicating that the antagonism is surmountable. The antagonistic potency of the analogue is greater in the coronary vessels than in heart muscle. This has been shown by calculating pA_2 values of the analogue against the effects of A II on coronary perfusion pressure and on the contractility of heart muscle. In coronary vessels the pA_2 value is 9·24, a value which is significantly higher than that obtained in heart muscle ($P < 0{\cdot}001$) and is almost equal to the value obtained in rabbit isolated aorta (Türker & others, 1972). These results suggest that the receptor sites in coronary vessels and rabbit aorta may be similar, since the pA_2 values are the same (Schild, 1947). The receptor sites in the myocardium may perhaps be different since the pA_2 value in heart muscle is lower than those values observed in coronary vessels or rabbit aorta. Conversely, the duration of the antagonistic effect is longer in the heart muscle than it is on the coronary vessels. Under similar conditions the duration of the antagonistic effect of AIA II, which is another 8-substituted analogue of angiotensin and structurally related to SIA II, is found to be significantly shorter. The only structural difference between these two analogues is that sarcosine is replaced with aspartic acid in position 1.

A difference in the rate of metabolism of the antagonists would affect the concentration reaching the receptor site. We previously reported that the duration of the

antagonistic effect of SIA II is longer than AIA II, even though their pA_2 values are similar (Yamamoto & others, 1972; Türker & others, 1972). This difference in duration appears to be due to structural change in the *N*-terminal position of the analogue. We have assumed that sarcosine in the one position protects the analogue from degradation, allowing it to be available to the receptor site for longer periods of time. Another possibility is that the enzyme systems which degrade SIA II are present in lower concentrations in heart muscle than in the wall of the coronary vessels, therefore, the analogue would again be available to the receptor in heart muscle for longer periods of time.

SIA II appears to be a specific antagonist of A II since it does not inhibit the effect of noradrenaline, vasopressin, bradykinin, histamine, prostaglandins and acetylcholine in either coronary vessels or in heart muscle. It has been suggested that a large part of the inotropic effect of A II is due to release of catecholamines from adrenergic neurons (Beaulnes, 1963). However, this has not been confirmed by Fowler & Holmes (1964) or Koch-Weser (1964). In the present study, the interaction of A II and the adrenergic neuron does not play a significant role on the mechanism of the positive inotropic effect or on the constrictor effect of the octapeptide in coronary vessels. This has been shown by the fact that the α-adrenoceptor blocker did not change the constrictor response to A II in the coronary vessels, nor did the β-adrenoceptor blocker inhibit the inotropic effects.

The present experiments show that A II has a specific receptor system in heart muscle since a specific competitive inhibitor blocks the vasoconstrictor as well as the myotropic effects of the peptide (Türker & others, 1972). Further, preliminary results also indicate that this analogue inhibits the ganglionic receptors of A II (unpublished observations).

From these studies as well as from our previous results (Türker & others, 1974), it can be concluded that angiotensin receptors are present in smooth muscle, in sympathetic ganglia and in heart muscle since the 8-substituted analogues specifically antagonize the agonistic effect of A II. These results also confirm our previous findings (Türker & others, 1972) that SIA II is a long-lasting and potent competitive inhibitor of A II.

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