Partial inhibition of prostaglandin-induced contraction of the rat colon by analogues of angiotensin II

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Substitution of the phenyl group of the angiotensin molecule or only a change of the spatial orientation of the phenyl group by substituting D-Phe for Phe has provided analogues which antagonize the action of angiotensin II and angiotensin I in vitro (Khairallah et al 1970; Gagnon et al 1971; Regoli et al 1974) as well as in vivo (Regoli & Park 1972).

Receptors for angiotensin appear to be specific and similar in rat intestinal (rat stomach strip and rat colon) and in rabbit vascular (rabbit aorta) smooth muscles (Regoli et al 1974). The specificity of analogues of angiotensin II which act as antagonists has been examined extensively only on rat stomach strip and rabbit aorta because these two tissues are devoid of spontaneous activity.

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The present experiments extend the study of angiotensin antagonists to the myotropic action induced by prostaglandins E_2 and $F_{2\alpha}$ as well as angiotensin II on the rat ascending colon.

Segments of rat ascending colon (3–4 cm) were superfused (10 ml min^{-1}) with oxygenated (95% O_2, 5% CO_2) Krebs solution at 37 °C. Responses to angiotensin II and prostaglandins E_2 (PGE_2) and $F_{2\alpha}$ ($PGF_{2\alpha}$) were recorded before and during continuous infusion of antagonists. Solutions of agonists were infused into the superfusing fluid at a rate of 0·1 ml min^{-1} for prostaglandins (5 and 10 ng ml^{-1}), and 1 ml min^{-1} for angiotensin (0·5 and 1·0 ng ml^{-1}), and the responses were recorded isotonically with a Harvard smooth muscle transducer on a Harvard recorder. Antagonists were infused (1, 10 and 100 ng ml^{-1}) at 0·1 ml min^{-1} for 15 to 30 min (until the tissues recovered their base lines) before second infusion of agonist commenced.

The following drugs were used: angiotensin II amide (Ciba Ltd, Dorval); [8-Leu] -angiotensin II, [1-Sar, 8-Leu]-angiotensin II, [8-Gly]-angiotensin II and [8-L-

Table 1. Percent inhibition of the myotropic action of PGE_2, $PGF_{2\alpha}$ and ATII on the rat ascending colon by different concentrations (expressed as ratios agonist/antagonist) of four C-terminal-substituted analogues of angiotensin II.

		Ratio Agonist/Antagonist					
Antagonists	Agonists	0·005	0·01	0·05	0·1	0·5	1·0
	PGE_2	—	—	40·5 ± 7·9 (8)	59·9 ± 6·4 (8)	57·5 ± 8·4 (9)	58·1 ± 8·1 (9)
[8-Leu] ATII	$PGF_{2\alpha}$	—	—	35·9 ± 11·4 (8)	31·0 ± 8·7 (8)	44·7 ± 9·3 (9)	49·4 ± 7·3 (9)
	ATII	100 ± 0 (8)	100 ± 0 (8)	93·7 ± 4·4 (9)	97·8 ± 1·7 (9)	2·8 ± 2·7 (12)	4·6 ± 2·9 (12)
	PGE_2	—	—	51·6 ± 5·8 (11)	49·8 ± 6·6 (11)	52·9 ± 12·6 (8)	55·9 ± 9·7 (8)
[1-Sar, 8-Leu] -AII	$PGF_{2\alpha}$	—	—	42·4 ± 7·3 (11)	44·4 ± 5·6 (11)	41·1 ± 10·0 (8)	41·5 ± 8·8 (8)
	ATII	—	—	83·9 ± 11·4 (8)	96·1 ± 2·8 (8)	0 ± 0 (12)	5·1 ± 2·6 (12)
	PGE_2	—	—	40·6 ± 5·3 (12)	45·7 ± 5·5 (12)	42·7 ± 9·2 (12)	36·2 ± 9·4 (12)
[8-Gly] -ATII	$PGF_{2\alpha}$	—	—	25·2 ± 5·0 (12)	30·5 ± 3·6 (12)	26·4 ± 3·6 (12)	24·0 ± 6·0 (12)
	ATII	85·1 ± 2·1 (12)	81·2 ± 2·7 (12)	23·5 ± 6·7 (12)	22·8 ± 5·5 (12)	0·4 ± 0·4 (6)	1·5 ± 1·4 (6)
	PGE_2	—	—	42·4 ± 9·8 (10)	44·4 ± 8·3 (10)	—	—
[8-L-Ala] -ATII	$PGF_{2\alpha}$	—	—	39·3 ± 9·0 (10)	37·7 ± 7·6 (10)	—	—
	ATII	95·6 ± 3·0 (10)	92·3 ± 3·4 (10)	—	—	—	—

Figures in parentheses are the number of observations.
Each percentage is a mean ± s.e. mean.

Ala]-angiotensin II (synthesized and kindly supplied by the late Dr. W. K. Park of this department); prostaglandins E_2 and $F_{2\alpha}$ (Upjohn Co., Kalamazoo, U.S.A. and Ono Pharm., Japan).

Responses were calculated as area with a Keuffel & Esser planimeter and expressed as mean percentage of inhibition ± s.e. mean.

Table 1 shows the percentages of inhibition produced by four analogues of angiotensin II expressed as ratios of agonist/antagonist. [8-Leu]-angiotensin II and [1-Sar, 8-Leu]-angiotensin II were the most potent antagonists against the parent molecule since the action of angiotensin was nearly completely abolished at a ratio as high as 0·1. A higher concentration of [8-Gly]-angiotensin II was required to antagonize the action of the octapeptide since at ratios of 0·05 and 0·1 the percentages of inhibition were only 23 and 22% respectively, whereas at higher concentrations (ratios: 0·005 and 0·01), [8-Gly]-angiotensin II produced a good antagonism (85 and 81%, respectively). At the same ratios (0·005 and 0·01), [8-L-Ala]-angiotensin II also exhibited a high activity (95 and 92% inhibition). None of the antagonists however caused inhibition of angiotensin with higher ratios of agonist/antagonist than 0·5 and 1·0.

The action of these four angiotensin antagonists on the myotropic action of prostaglandins E_2 and $F_{2\alpha}$ gave a completely different pattern. Responses to PGE_2 and $PGF_{2\alpha}$ were inhibited by ratios as high as 1·0. However even when this dose of antagonist was increased to give a ratio of 0·05 the extent of inhibition was about the same. The percentages of inhibition of PGE_2 vary from 40 to 59% and of $PGF_{2\alpha}$ from 31 to 49%.

These results confirm previous studies (Gagnon et al 1971; Gagnon & Sirois 1972; Regoli et al 1974) on the potency of analogues of angiotensin II to inhibit the myotropic action of angiotensin II on the rat colon and show that a full inhibition of the responses elicited by different concentrations of the octapeptide needs concentrations of antagonists at least 10 times greater with [8-Leu]-angiotensin II and [1-Sar, 8-Leu]-angiotensin II and about 100 times greater with [8-Gly]-angiotensin II.

Although the specificity of angiotensin antagonists has been clearly demonstrated on rat stomach strip and rabbit aorta (Park et al 1973; Regoli et al 1974), the present experiments show that the responses of the rat colon to prostaglandins E_2 and $F_{2\alpha}$ are partially inhibited by the analogues. However, two findings suggest that the prostaglandin antagonism is not a specific receptor interaction. Firstly, although the potency of the antagonists varies considerably against the responses to angiotensin, their activity is almost the same in respect of responses to prostaglandins. Secondly, the antagonism of prostaglandins is not dose-dependent as with angiotensin.

We conclude, therefore, that although 8-Phe substituted analogues of angiotensin cause partial inhibition of PGE_2 and $PGF_{2\alpha}$, they nevertheless still appear to be specific competitive antagonists.

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