Short communications

Specific antagonists for the myotropic action of angiotensin II and angiotensin I on the isolated rat colon

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Substitutions of 8 phenylalanine with L-alanine and D-phenylalanine abolish the myotropic action of the angiotensin II (AT_{II}) analogues and confer inhibitory properties on the molecule. [8-L-Ala]-AT_{II} and [8-D-Phe]-AT_{II} antagonize specifically the myotropic action of AT_{II} and angiotensin I (AT_I) on the rat colon, while the action of other myotropic agents (acetylcholine, 5 hydroxytryptamine) is not modified.

Substitution of an unnatural amino-acid (Acpc: 1-aminocyclopentane carboxylic acid) for each of the eight amino-acids composing the angiotensin molecule has provided the evidence that phenylalanine in position 8 is essential for the myotropic activity of angiotensin II (AT_{II}) and angiotensin I (AT_I) (Regoli & Park, 1971a). This finding confirms the observation of other authors (Khairallah, Toth & Bumpus, 1970).

Elimination of phenyl group by substituting Ala for Phe in position 8 or change of the spatial orientation of the phenyl group, by substituting D-Phe for Phe, has provided analogues of angiotensin which antagonize specifically the action of AT_{II} and AT_I *in vivo* (Regoli & Park, 1971a). The purpose of this investigation was to study the effect of the two angiotensin analogues on the myotropic effect of AT_{II}, AT_I and other smooth muscle stimulating agents on the isolated rat colon.

Methods.—Segments of ascending rat colons (3–4 cm) were superfused with oxygenated (CO_2–5%, O_2–95%) Krebs solution according to the technique of Regoli & Vane (1964), as described by Belisle & Gagnon (1971). Agonists were infused for fixed periods of 4 min into the circuit superfusing the isolated rat colons and the contractions were recorded isotonically with a Harvard smooth muscle transducer on a Harvard recorder. Changes of the base line were calculated with a Gelman planimeter, and the results expressed as the area below the tracing.

The following substances were used: AT_{II} (Val_5-angiotensin II, Hypertensin, Ciba), AT_I (Ile_5-angiotensin I, synthesized by Dr. W. K. Park), acetylcholine HCl and 5-hydroxytryptamine creatinine sulphate (serotonin) (Sigma), [8-L-Ala]-AT_{II} and [8-D-Phe]-AT_{II}, synthesized in our laboratory (Regoli & Park, 1971b).

Dose-response curves of agonists were measured before, during and after infusion of [8-Ala]-AT_{II} or [8-D-Phe]-AT_{II}. The antagonists were infused for 30 min to measure the direct effect on the isolated rat colon and to obtain stable concentrations on the tissue before testing the activity of agonists. Doses are expressed in ng/ml of perfusing fluid and concentrations of base have been calculated for the non-peptide agonists. Results are given as means ± S.E. of the response elicited by the agonist in the absence and in the presence of antagonist. Significance of differences has been calculated with the *t* test for paired data.

Results.—At the doses used [8-L-Ala]-AT_{II} and [8-D-Phe]-AT_{II} evoked a weak and unsustained contraction of the preparation; in both cases the base line came back to control levels despite their presence in the superfusing medium.

Table I summarizes the effect of [8-Ala]-AT_{II} and [8-D-Phe]-AT_{II} on the myotropic action of AT_{II}, AT_I, ACh and 5-HT. In the presence of [8-Ala]-AT_{II}, the response of the isolated rat colon to AT_{II} and AT_I decreased by about 90%, while that to acetylcholine and 5-hydroxytryptamine was not significantly modified. [8-D-Phe]-AT_{II} had similar effect on AT_{II} and AT_I and decreased slightly the response to 5-HT: acetylcholine was not influenced. The doses of agonists reported in Table 1 have been chosen on the basis of the extent of contractions and reflect different sensitivity of the isolated rat colon to these four agonists.

In some experiments, after observing the effect of the antagonists, control Krebs solution was again superfused and the activity of all agonists tested 30 min later. Under these circumstances, AT_{II} and AT_I regained their normal myotropic activity,

TABLE 1. *Effect of some analogues of angiotensin II upon the myotropic action of angiotensin II, angiotensin I, acetylcholine and 5-hydroxytryptamine on the isolated rat colon*

Antagonist	Agonist	Dose of agonist (ng/ml)	N	Response (cm^2) Before antagonist	After antagonist	P
[8-L-Ala]-AT_{II} (0·25 μg/ml)	Angiotensin II	0·1	21	3·14±0·70	0·59±0·14	<0·01
	Angiotensin II	0·3	21	10·17±1·08	1·10±0·24	<0·001
	Angiotensin II	1·0	21	19·11±1·02	1·96±0·45	<0·001
	Angiotensin I	10	21	7·97±1·03	1·36±0·30	<0·001
	Angiotensin I	30	21	15·85±1·16	2·91±0·49	<0·001
	Acetylcholine	3	18	6·39±1·27	6·51±1·02	N.S.
	Acetylcholine	10	18	15·05±1·94	17·16±2·01	N.S.
	5-hydroxytryptamine	100	18	9·50±1·61	10·71±1·67	N.S.
	5-hydroxytryptamine	300	18	11·41±1·65	14·04±1·87	N.S.
[8-D-Phe]-AT_{II} (1 μg/ml)	Angiotensin II	0·3	22	12·92±0·71	0·64±0·10	<0·001
	Angiotensin II	0·5	22	16·39±1·01	1·43±0·21	<0·001
	Angiotensin I	10	14	8·11±1·15	1·60±0·73	<0·001
	Angiotensin I	30	14	17·01±1·47	3·54±1·10	<0·001
	Acetylcholine	3	22	5·86±0·72	5·69±0·83	N.S.
	Acetylcholine	10	22	14·91±1·24	15·25±1·16	N.S.
	5-hydroxytryptamine	100	22	10·01±1·42	7·23±1·35	N.S.
	5-hydroxytryptamine	300	22	13·45±1·37	11·20±1·34	N.S.

N=number of preparations. Responses are expressed as the mean ± standard error. N.S. signifies that the responses evoked before and after the antagonist are not significantly different.

showing that the antagonistic action of both [8-Ala]-AT_{II} and [8-D-Phe]-AT_{II} was reversible.

Discussion.—The results obtained with [8-Ala]-AT_{II} confirm previous studies (Khairallah *et al.*, 1970) on guinea-pig ileum. However, angiotensin seems to have both a direct myotropic and a cholinergic action on the guinea-pig ileum. The isolated rat colon is apparently a more specific preparation than the guinea-pig ileum, because angiotensin has only a direct myotropic action. By increasing the dose of [8-Ala]-AT_{II} or [8-D-Phe]-AT_{II}, a complete block of the action of AT_{II} and AT_{I} can be obtained on this preparation.

Our experiments suggest that specific antagonists against AT_{II} and AT_{I} may be obtained by changing the spatial orientation or by removing the phenyl group of 8-Phe. The antagonism is specific for AT_{II} and AT_{I}, while myotropic actions of acetylcholine and 5-hydroxytryptamine are not influenced.

The authors wish to thank M. P. Boucher for his technical assistance and Mme C. Audet for typing the manuscript. D.J.G. is a scholar of the Medical Research Council of Canada. This work was supported by the grants no. MT-4032 and no. MA-3617 of the Medical Research Council of Canada.

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(*Received June*, 14, 1971)