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The rat isolated colon as a specific assay organ for angiotensin

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Summary

1. The antagonism of the contractile effects of prostaglandins $F_{2\alpha}$ and E_2 by polyphloretin phosphate (PPP) was studied on the rat isolated colon.

2. PPP (20 μg/ml) was found to reduce the myotropic action of angiotensin I (10 ng/ml) and II (0·3 ng/ml) whereas larger doses of both agents were not influenced.

3. It is concluded that when a mixture of antagonists is used, including PPP, the rat isolated colon can be used to assay angiotensin with accuracy and specificity. The addition of 8-L-ala-AT_{II} affords a further test of specificity.

Introduction

Angiotensin can be assayed on various preparations of isolated smooth muscle, especially the rat ascending colon (Regoli & Vane, 1964; Cohn & Notargiacomo, 1969; Lemieux & Regoli, 1971). Although the rat colon exhibits a very high sensitivity to angiotensin, its use as an assay organ is limited by a lack of specificity when other naturally-occurring substances are present in the sample tested.

The purpose of the present study was to use Gaddum's superfusion technique (1953) for the assay of angiotensin on the rat ascending colon which was rendered specific to this substance by the use of a mixture of antagonists. Furthermore, 8-L-ala-angiotensin II, a specific inhibitor of angiotensin (Gagnon, Park & Regoli, 1971; Türker, Yamamoto, Khairallah & Bumpus, 1971) has been used to confirm the presence of angiotensin in bioassay samples.

Methods

The technique of superfusion has been described in detail previously (Belisle & Gagnon, 1971). In brief, segments of rat ascending colon (3–4 cm) were superfused (10 ml/min) with oxygenated (5% CO_2, 95% O_2) Krebs solution at 37° C. Solutions of agonists were infused through a rubber tube into the superfusing fluid at a rate of 0·1 ml/min except for angiotensin I and II (1 ml/min), and the responses recorded isotonically with a Harvard smooth muscle transducer on a Harvard recorder. The antagonists were infused (0·1 ml/min) 15 to 20 min before the administration of agonists and kept in contact with tissues for the remainder of the experiment. All drug solutions were freshly prepared in Krebs, and adrenaline was protected against oxidation by the addition of ascorbic acid (0·3 mg/ml). The concentration of drugs always refers to the final concentration of the free bases (except for peptides) in the superfusing fluid. The following drugs

were used: (−)-adrenaline bitartrate, 5-hydroxytryptamine creatinine sulphate, acetylcholine chloride, atropine sulphate (Sigma Chemical Co., St. Louis, U.S.A.); oxprenolol hydrochloride, phentolamine hydrochloride and angiotensin II amide (Ciba Co. Ltd., Dorval); methysergide bimaleate (Sandoz, Montreal); polyphloretin phosphate (PPP), AB LEO, Halsingborg, Sweden); prostaglandins E_2 and $F_{2\alpha}$ (Upjohn Co., Kalamazoo, U.S.A.); 5-Ile-angiotensin I and 8-L-ala-angiotensin II (synthesized by Dr. W. K. Park in our department).

Changes in the base line were calculated with a Keuffel & Esser planimeter and expressed as the area below the tracing. Results are given as means ± S.E. of the response elicited by the agonists in the absence and in the presence of antagonists. Significance of differences was calculated with the *t* test for paired data.

Results

In a first series of experiments (35 observations) we studied the direct effects of a mixture of inhibitors upon the preparation as well as its antagonistic properties upon the responses of the rat colon to different agonists. The mixture of antagonists contained oxprenolol (10 μg/ml), phentolamine (1 μg/ml), atropine (1 μg/ml) and methysergide (0·5 μg/ml); 8-L-ala-angiotensin II (0·25 μg/ml), a specific and competitive inhibitor of angiotensin, was administered separately.

Following its infusion into the superfusing fluid, the mixture of antagonists induced a rapid decrease in muscle tone and greatly diminished the spontaneous activity of the tissue allowing the base line to remain very stable for the rest of the experiment. Furthermore, in our experimental conditions, the antagonists abolished completely the responses of the rat isolated colon normally induced by adrenaline (10 and 30 ng/ml), acetylcholine (3 and 10 ng/ml), 5-HT (100 and 300 ng/ml), angiotensin I (10 and 30 ng/ml) and angiotensin II (0·1 and 0·3 ng/ml).

Prostaglandins of the E and F series induce contraction of the rat isolated colon, and may therefore interfere with the assay of angiotensin. The next series of experiments was designed to study the antagonistic effects of polyphloretin phosphate (PPP) upon the contraction of the rat colon induced by prostaglandins.

As can be seen from Table 1, there was a rather large degree of variation in the myotropic action of PGE_2 and $PGF_{2\alpha}$ at the doses used (1, 5 and 10 ng/ml);

TABLE 1. *Effect of different concentrations of polyphloretin phosphate (PPP) upon the contraction of the rat isolated colon induced by prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)*

Antagonist	Agonist	Dose of agonist (ng/ml)	*n*	Response (cm²) Before antagonist	After antagonist	*P*
PPP (10 μg/ml)	PGE_2	1	12	6·00±0·80	3·20±0·88	<0·05
	PGE_2	5	12	13·80±1·19	7·10±1·15	<0·001
	PGE_2	10	12	15·66±1·47	9·66±1·71	<0·02
	$PGF_{2\alpha}$	1	12	0·98±0·35	0·33±0·18	N.S.
	$PGF_{2\alpha}$	5	12	5·95±1·72	2·38±0·50	<0·001
	$PGF_{2\alpha}$	10	12	21·18±2·22	6·76±1·30	<0·001
PPP (20 μg/ml)	PGE_2	1	39	5·58±0·53	0·97±0·30	<0·001
	PGE_2	5	39	12·32±1·00	4·82±0·82	<0·001
	PGE_2	10	39	15·42±1·23	6·51±0·99	<0·001
	$PGF_{2\alpha}$	1	39	3·15±0·39	0·0	
	$PGF_{2\alpha}$	5	39	15·35±1·15	0·63±0·21	<0·001
	$PGF_{2\alpha}$	10	39	22·17±1·24	2·26±0·55	<0·001

n=Number of observations.

the preparation seemed to be more sensitive to the action of $PGF_{2\alpha}$, especially at high dose levels. When tested 15 min after the infusion of PPP (10 and 20 μg/ml) in the superfusing medium, both prostaglandins induced contractile effects which were significantly smaller than controls. From these experiments, it appears that PPP can be used in order to prevent the action of prostaglandins on the rat colon.

It has been shown previously that the antagonists used in our mixture do not interfere with the myotropic actions of angiotensin. In the present experiments the effect of the addition of PPP to the mixture was examined on the response of the rat isolated colon to angiotensin I and II.

As shown in Figure 1, following the addition of PPP (20 μg/ml), the mixture of antagonists ('After Mixture') reduced significantly ($P<0.05$) the effect of doses of 0·3 ng/ml of angiotensin II and 10 ng/ml of angiotensin I, whereas larger doses were not affected. The infusion of 8-L-ala-AT_{II} in the presence of the mixture of antagonists abolished completely or diminished significantly the responses to angiotensin I and II. Further studies showed that the residual effect of angiotensin II (3 ng/ml) can be completely prevented by increasing the dose of 8-L-ala-AT_{II}.

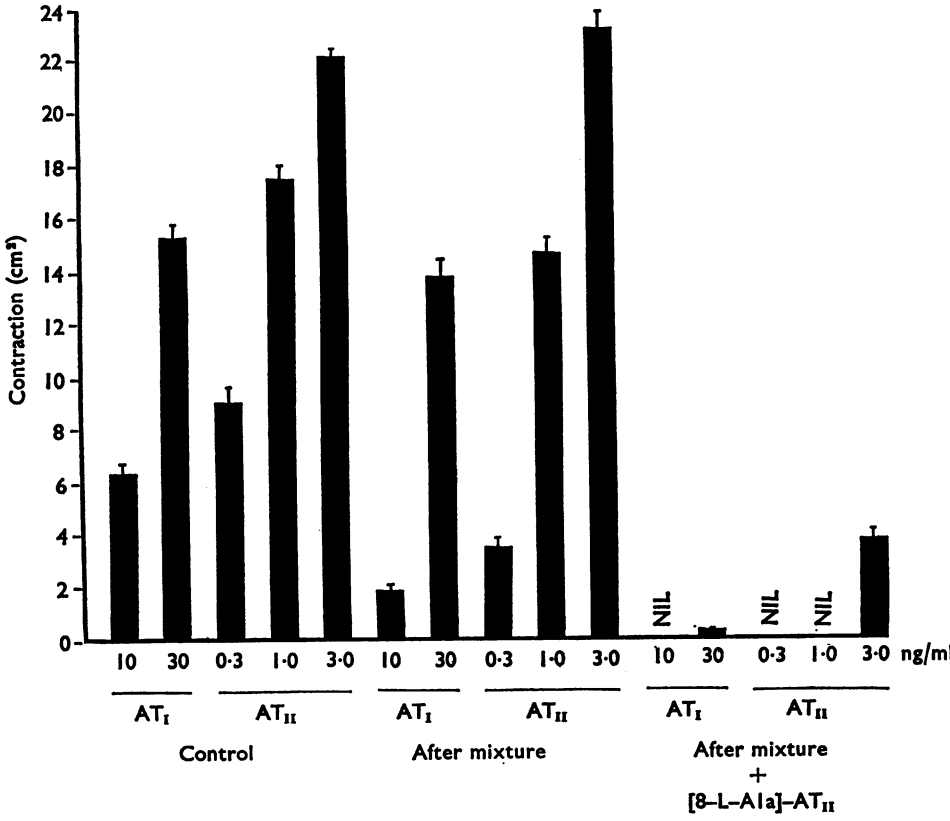

FIG. 1. Histogram showing the effects of different concentrations of angiotensin I and II upon the rat isolated colon, alone (control), in the presence of a mixture of inhibitors (after mixture) and finally in the presence of the mixture of inhibitors plus 8-L-ala-AT_{II} (0·25 μg/ml). The mixture of inhibitors contained (μg/ml): oxprenolol (10), phentolamine (1), atropine (1), methysergide (0·5) and polyphloretin phosphate (20). Vertical bars, S.E. of mean.

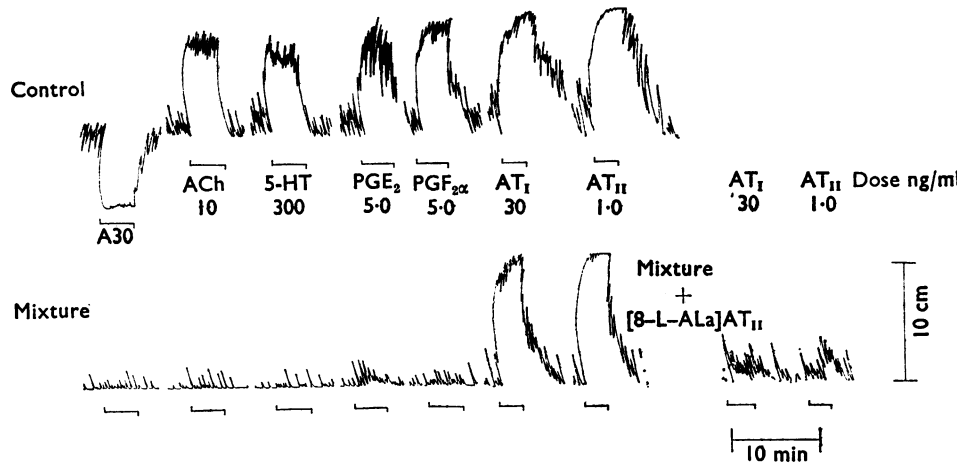

FIG. 2. Effects of the infusion of different agonists upon the rat isolated colon before (upper tracing) and after (lower tracing) the continuous administration of a mixture of inhibitors (mixture). The effect of the addition of 8-L-ala-AT_{II} is shown at the right of the lower tracing. The mixture of inhibitors is similar to that described for Fig. 1. Horizontal bars, period of infusion of agonists.

Figure 2 shows a typical experiment in which the mixture of inhibitors (including PPP, 20 μg/ml) was used to characterize the contractile action of angiotensin.

Control responses (upper tracing) were first obtained with all agonists. The mixture of antagonists was then infused into the superfusing fluid 15 min before the second infusion of agonists. In the presence of inhibitors (lower tracing) the responses to adrenaline, acetylcholine, 5-hydroxytryptamine and prostaglandins E_2 and $F_{2\alpha}$ were abolished whereas those to angiotensin I and II were unaffected. The angiotensin inhibitor, 8-L-ala-angiotensin II, was then added to the superfusing medium in order to prevent the contractile effect of angiotensins (lower tracing, extreme right) showing the specificity of the assay.

Discussion

The presence of naturally occurring substances in plasma or other biological fluids has always been a drawback in the use of the rat ascending colon as an assay organ for angiotensin. It is evident that the rat colon is much more specific than any other tissue to angiotensin (Regoli & Vane, 1964), but improved specificity can nevertheless be achieved with the use of antagonists. Our previous studies have shown that a mixture of α- and β-adrenoceptor blocking agents prevent the action of catecholamines (Gagnon & Belisle, 1970; Belisle & Gagnon, 1971); methysergide inhibits the stimulating action of 5-hydroxytryptamine (Gagnon, 1972); and in addition atropine antagonizes the action of acetylcholine and its derivatives.

The rat isolated colon responds to PGE_2 and especially to $PGF_{2\alpha}$ (Ferreira & Vane, 1967; Vane, 1971), but the present experiments have shown that polyphloretin phosphate (PPP) which is known to inhibit the responses to prostaglandins of the jird colon, rabbit jejunum (Eakins, Karim & Miller, 1970; Eakins, Miller & Karim, 1971), and of human, guinea-pig and rat gastrointestinal muscle (Bennett & Posner, 1971) significantly diminished or even abolished responses of

the rat colon to concentrations of prostaglandins likely to be found in plasma. Moreover, our results showed that the contractions of the rat colon induced by prostaglandin $F_{2\alpha}$ were more readily antagonized by PPP than were those to prostaglandin E_2; this finding is in agreement with previous reports with the jird colon (Eakins *et al.*, 1970). We also observed that PPP produces a slight reduction in the sensitivity of the assay organ to angiotensin. Nevertheless, this is not considered as a serious problem and can easily be compensated for by increasing the gain of the recording system.

Apart from preventing the unwanted effects of other naturally occurring substances, the mixture of inhibitors has also the advantage of greatly diminishing or even abolishing completely the spontaneous activity of the assay organ. Furthermore, it stabilizes the base-line so that it becomes very easy to measure any response to angiotensin.

Since the rat ascending colon is relatively insensitive to substances other than angiotensin, likely to be found in blood, for instance, histamine, oxytocin, ADH, bradykinin (Vane, 1969), angiotensin may now be assayed in biological fluids with accuracy and specificity. The specific inhibitor, 8-L-ala-angiotensin II (Gagnon *et al.*, 1971) may be used as final proof that the substance being assayed is angiotensin.

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