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EVALUATION AS A BIOASSAY PREPARATION OF TORTOISE INTESTINE FOR PROSTAGLANDIN E_1

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Summary

The isolated strips of tortoise intestine are evaluated as a test organ for bioassay of prostaglandin E_1. This preparation responded highly sensitively to PGE_1 and PGE_2 in picogram concentration range. The mean slope and the value of precision index among the doses of 0.1, 0.3 and 0.5ng/ml in final concentration were 37.7 and 0.143, respectively. And this was relatively insensitive to different prostaglandins; $E_1/E_2 \gtrsim 1$, $E_1/A_2 \sim 50$ and $E_1/F_{2\alpha} \sim 100$, and showed the dual responses to 5-hydroxytryptamine and histamine; initial contraction followed by relaxation. The dose-ratio inducing the relative equal contraction height for PGE_1, acetylcholine, caerulein, angiotensin and barium chloride was 0.4 : 50 : 25 : 10 : 100 in this order. These results suggest that the intestinal strips of the tortoise are suitable for bioassay of prostaglandin E_1 and E_2 between the doses of 0.1 and 1.0 ng/ml level in the tissue extracts.

INTRODUCTION

A large number of assay preparations for prostaglandins(PGs) and their metabolites have been investigated because bioassay technique is still widely used as an important tool in the study of physiological roles of these substances. The common preparations most widely used are rabbit duodenum, guinea-pig ileum, hamster and gerbil colon, chicken rectum and rat stomach fundus. The status of bioassay for PGs has recently been compiled in the review(1). And recently, the value of the hamster stomach was reported as an assay preparation for PGE_2(2). The sensitive physicochemical methods for quantitative determination and separation have been devised for estimation of PGs(3) and even though gas-liquid chromatography, mass spectrometry may eventually replace these methods, the simple and convenient bioassay with gastrointestinal segments is highly sensitive enough to permit quantitative assays at nanogram per milliliter level.

In the study of actions of PGs on the intestinal motility of various animals in viewpoint of genera differences, phylogenetically, it was found that the longitudinal strips of tortoise intestine (Amyda japonica) was highly sensitive to PGE_1 and PGE_2(4). It was, therefore, of interest to evaluate the intestine of tortoise as a bioassay preparation and to consider some problems occurred.

METHODS

Animals

1. Tortoise (300~400 g body weight, Amyda japonica) were fixed on their back on the board and killed by severing the cervical spine with a bone cutter. The upper and middle portions of intestine (about 35 mm length) was removed and suspended in the isolated organ baths containing 15 ml Frog-Ringer solution (NaCl 6.4, KCl 0.3, $CaCl_2$ 0.18, $MgCl_2$ 0.01, $NaHCO_3$ 0.3, and glucose 2.0 g/liter), bubbled with oxygen at room temperature. This experiment was carried out from May to September.

2. Guinea-pig terminal ileum and the fundic portion of hybrid rat stomach (5) were mounted in an organ bath containing 15 ml Tyrode solution at 37°C and gassed with oxygen.

Experiment

The muscle activity was recorded with an isotonic lever (magnified by 10 fold) writing on a smoked drum. Each muscle strip mounted was allowed to be stabilized for 30~60 minutes, thereafter, acetylcholine was added to see the responsiveness of the preparation to the stimulants. If the contraction height to 10^{-7} g/ml acetylcholine was less than 30 mm in height, then it was discarded. And the preparations were treated with 10^{-6} g/ml atropine for 20 minutes and washed out with working solution.

Drugs

Drugs used in this experiment are acetylcholine HCl (Sigma), atropine sulfate(Sigma), 5-hydroxytryptamine creatinine sulfate(serotonin, Sigma), histamine HCl (Sigma), caerulein(Farmitalia, F. I. 6934, caerulein), angiotensin (Ciba), $BaCl_2$(Mallinckrodt), cocaine HCl(U.S. P.) and prostaglandins (Upjohn). PGs(1 mg) were dissolved in 95% ethanol(0.5 ml) and whenever diluted, the aqueous solution was prepared with 0.02% w/v Na_2CO_3 in 0.9% aqueous NaCl.

RESULTS

The intestinal strips of tortoise contracted slowly with a high degree of sensitivity even to smaller amounts of PGE_1 and PGE_2 at least by 20~40 picogram per milliliter (Figure 1). The contraction started 10~15 second after the addition of the agonist and reached maximum in 40~50 second and did not relax spontaneously. After washing out the tension slowly returned to its baseline within 4~5 minutes, so that the sufficient time was permitted for complete relaxation.

The dose-response curve of tortoise intestine to PGE_1 greatly shifted to the left, comparing with the that of the guinea-pig ileum and the rat stomach fundus strips (Table 1 and Figure 2). Its dose-ratio of 50% of maximum contraction of tortoise to guinea-pig ileum for PGE_1 was nearly 0.029 (the dose needed indu-

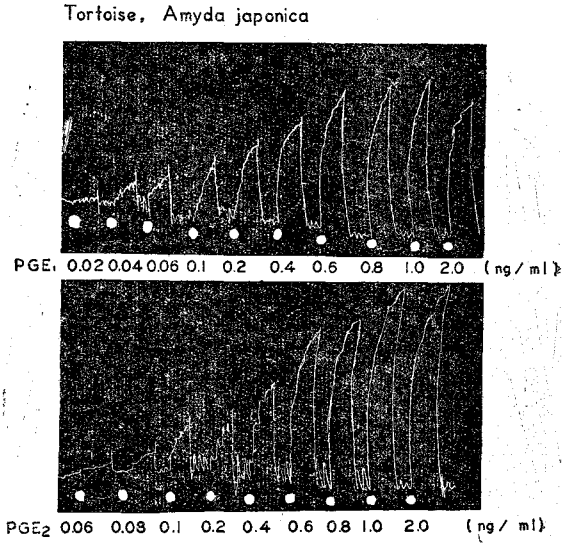

Fig. 1. Tracing of dose-dependent responses of tortoise intestine to PGE_1 and PGE_2.

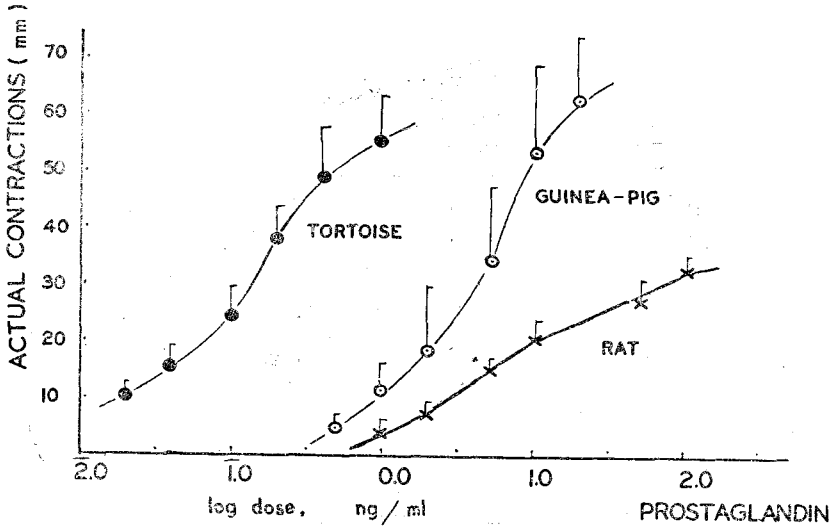

Fig. 2. Dose-response curves for PGE_1 of the three isolated smooth muscles.

Table 1. Concentration-effect relationships for PGE_1 on the three isolated smooth muscles.

Tortoise small intestine (N, 7)		Guinea-pig ileum (N, 7)		Rat stomach fundus (N, 7)	
ng/ml	Mean±S.D. (mm)	ng/ml	Mean±S.D. (mm)	ng/ml	Mean±S.D. (mm)
0.02	10.7± 3.0(1)*	0.5	4.4± 2.6	1	4.3±2.1
0.04	15.7± 4.3	1.0	11.0± 3.7	2	8.4±1.3
0.10	24.6± 5.6	2.0	17.6±13.1	5	14.0±2.7
0.20	38.6± 5.7	5.0	35.0±13.2	10	20.3±3.5
0.40	48.7± 9.6	10.0	53.6±15.5	50	25.6±3.4
1.00	56.6± 7.7	20.0	63.4±11.8	100	33.0±3.5
Mean	32.48	Mean	30.83	Mean	17.60
Acetylcholine response (10^{-6} g/ml)	97.6±22.0	Acetylcholine response (10^{-8} g/ml)	98.0±23.7	Acetylcholine response (10^{-6} g/ml)	21.6±9.8
Slope(2)*	28.92	Slope	38.71	Slope	13.67
Precision index	0.253	Precision index	0.227	Precision index	0.310

(1) Values are mean millimeters of pen deflections for the contractions of smooth muscles.
(2) Slopes are expressed as millimeters per unit of log concentrations.

cing 50 % of maximum contraction of tortoise intestine was divided by that of guinea-pig). Above the dose of 1 ng/ml PGE_1, the contraction height was not further increased. The guinea-pig ileum also responded very sensitively to PGE_1 rather than those of rat stomach fundus did. The concentration-effect relationships for PGE_1 on the isolated smooth muscles of tortoise, guinea-pig and rat were analyzed statistically (6, 7). Here each preparation was exposed to the six doses as an increasing dose-program, thereafter, slopes and precision index were calculated from each system. The slopes from linear regression for PGE_1 on the tortoise, guinea-pig

Table 2. Discriminating capacity of the small intestine of tortoise to different concentrations of PGE_1

Dose	Response, Actual contraction(Mean±S.D.), mm				
ng/ml log	A(N, 22)(1)*	B(N, 21)	C(N, 20)	D(N, 26)	Mean(N, 4)(2)*
0.1 −1	13.8± 5.7 (6)	13.0± 3.2 (7)	5.7± 1.9 (6)	8.2± 2.6 (6)	10.2± 3.9
0.3 −0.5229	28.2± 7.9 (6)	21.6± 3.9 (7)	17.0± 2.8 (6)	18.7± 3.3(10)	21.4± 4.8
0.5 −0.3010	46.5±13.0(10)	35.7± 3.4 (7)	30.3± 5.1 (8)	37.7± 8.6(10)	37.5± 7.1
Sample regression	Y=45.0X+56.9	Y=31.4X+42.5	Y=51.0X+48.6	Y=39.9X+45.8	Y=37.7X+46.0(3)*
Index of precision(λ)	0.183	0.242	0.104	0.094	0.143
Standard deviation of regression coefficient	7.60	6.89	7.99	3.17	5.88
95% Confidence limits of slopes	$32.7<\beta<57.3$	$24.1<\beta<38.7$	$42.5<\beta<59.5$	$29.6<\beta<50.1$	$31.1<\beta<44.3$

(1) Three different doses of PGE_1 were applied in random order for the number of brackets in one preparation of each tortoise and N means the total applications.
(2) Four preparations from each other tortoise were used.
(3) In this experiment, the preparations were previously treated with 10^{-6} g/ml atropine and cocaine and stored at 4°C Frog-Ringer solution for 1 to 2 hours to reduce the spontaneous movement.

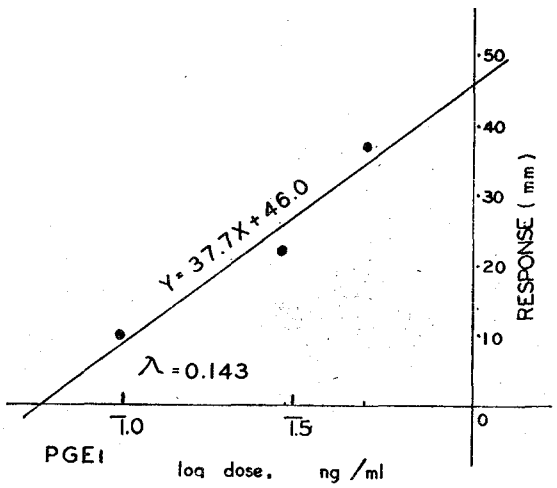

Fig. 3. A linear regression of the responses of tortoise intestine to three different concentrations of PGE_1 on the logarithmic scale, see others in the table 2.

ileum and rat stomach fundus were 28.92, 38.71 and 13.67, respectively and the index of precision were 0.253, 0.227 and 0.310 in the same order. It might be presumed that the responsiveness of the hybrid rat stomach funds strips

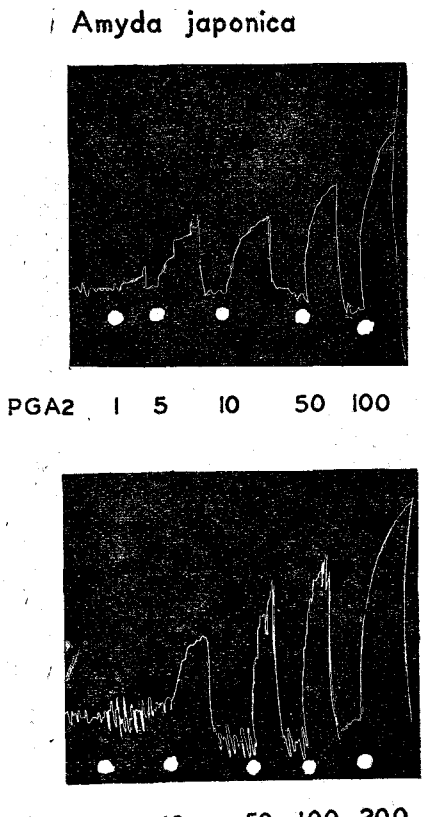

Fig. 4. Effects of PGA_2 and $PGF_{2\alpha}$ on the tortoise intestine.

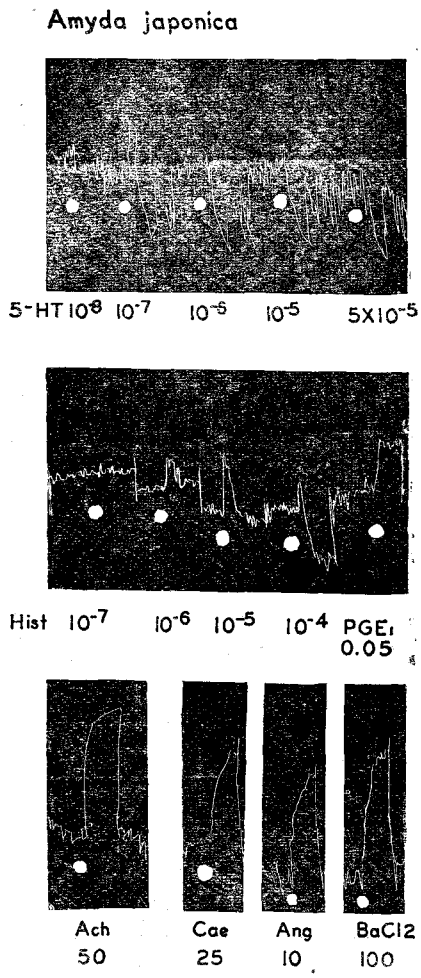

Fig. 5. Effects of several stimulants on the tortoise intestine. 5-HT; 5-hydroxytryptamine, Hist; histamine (g/ml), and Ach; acetylcholine, Cae; caerulein, Ang; angiotensin(ng/ml).

for PGs is much different from other strains of rat(8).

To test the discriminating capacity of tortoise intestine, the muscle strips were treated with 10^{-6} g/ml atropine and cocaine and stored at 4°C Frog-Ringer solution for 1 to 2 hours to reduce the spontaneous movement(9, 10). Four preparations from each other tortoise were selected and three different doses of PGE_1 were applied in random order, alternatively, to each muscle strips (Table 2). The mean slope and index of precision were obtained as follows: 37.7 and 0.143, respectively and the standard deviation of regression coefficient was 5.88 (Figure 3 showing a linear regression of response on the logarithmic concentration scale).

The tortoise intestinal strips showed the following sensitivity to different prostaglandins: $E_1/E_2 \gtrsim 1$, $E_1/A_2 \sim 50$ and $E_1/F_{2\alpha} \sim 100$ (Figure 4).

Other substances which may act as contaminants were tested. This preparation revealed the dual responses to 5-hydroxytryptamine and histamine with relative insensitivity, namely the initial transitory contraction followed by relaxation. When the doses needed to induce the nearly equal contraction height were compared, the ratio of PGE_1, acetylcholine, caerulein, angiotensin and barium chloride was 0.4 : 50 : 25 : 10 : 100 in this order (Figure 5).

DISCUSSION

As an assay preparation, the intestinal segments of tortoise have two disadvantages: One is that as the tortoise is cold-blooded animal the experiment has to be carried out in warm temperature from spring to early fall because the sensitivity to PGs was lessened during the winter season(11). The other is the spontaneous movement, which can be reduced by pretreatment with atropine and cocaine and storing it in the refrigerator(4°C) for 1 to 2 hours. Others reported that this was suppressed by reducing temperature or the calcium concentration of the bathing fluid(10), or by the addition of catecholamines(12), but the latter method should not be used for the tortoise intestine because it responded with contraction to norepinephrine or epinephrine(13).

This preparation also has several advantages,

that is, it contracted highly sensitively and specifically to PGE_1 and PGE_2 at picogram concentration range and the precision index was uniformly (λ=0.143) low and it is similar to the figures reported for PGE_1 on four mammalian gastrointestinal segments for the rat stomach(0.165), rabbit duodenum(0.51), guinea-pig ileum(0.255) and gerbil colon(0.115) (8), but not much better than the data for PGE_2 on the hamster stomach(λ=0.057).

There was little evidence of significant fatigue or tachyphylaxis by applying the agonist for 20 to 30 times successively and base-lines were satisfactorily sustained if sufficient time was allowed for complete relaxation. It was less sensitive to $FGF_{2\alpha}$ and PGA_2, and it reacted to 5-hydroxytryptamine, histamine and angiotensin with less sensitivity, so that it can be readily used to differentiate between PGE compound and other types of PGs, or biogenic amines discussed above.

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