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The use of the hamster stomach *in vitro* as an assay preparation for prostaglandins

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Summary

1. Hamster stomach strips *in vitro* are useful test preparations for the assay of prostaglandins E and F in the nanogram concentration range. Three-minute cycles can be used for long periods without spontaneous contractions or significant base-line instability.

2. The mean percentage error and the precision index of six 4-point assays of prostaglandin E_2 were 7·8 and 0·057, respectively.

3. The relative sensitivity to different prostaglandins was $E_2/E_1 \sim 1$, $E_2/F_{1\alpha} \sim 65$ and $E_2/A_1 \sim 400$.

4. These preparations were relatively insensitive to 5-hydroxytryptamine (prostaglandin E_2/5-HT$\sim$20,000) and histamine (prostaglandin E_2/histamine $\sim$200).

5. These results suggest that the hamster stomach preparation can be used to differentiate between 5-hydroxytryptamine and prostaglandins.

Introduction

Although sensitive and reliable physico-chemical methods have been devised for the characterization and estimation of prostaglandins in animal tissues and body fluids, bioassays are still widely used as important tools in the study of the physiological role of these lipids. A large number of assay preparations for prostaglandins and some of their active metabolites has been developed (Bergström, Carlson & Weeks, 1968 ; Shaw & Ramwell, 1969 ; Anggard, 1971 ; Horton, 1972). Among these, uterine and gastrointestinal segments isolated from different mammals are undoubtedly the most convenient preparations. They are both simple and sensitive enough to permit quantitative assays at nanogram concentrations. Moreover, the specificity of the bioassay can be improved by the use of inhibitors and by inserting a stage of purification by solvent partition and chromatography. Under these conditions, the biological assays are surpassed only by the reliable but expensive gas chromatography mass spectrometry method.

Experiments with strips cut from the pouch of the hamster stomach showed that the tissue responds by a fast and smooth contraction to prostaglandin E_2. This suggested that the new preparation could be used as a substitute for the rat stomach in the assay of prostaglandins. In the present study the usefulness of such preparations in the assay of nanogram quantities of prostaglandins was investigated.

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Methods

Adult male golden hamsters (*Mesocricetus auratus*) 120–150 g body weight were given water and food *ad libitum* until they were killed by decapitation. The stomach was removed, the pouch separated from the main body of the organ and washed in warm Krebs solution. Strips 5 to 7 cm long were then cut by making 3 to 5 incisions in the direction of the longitudinal muscle as is usually done for the rat stomach (Vane, 1957). The resulting strip was hung in an overflow organ bath (5 ml) at 37° C and the contractions recorded by an isotonic frontal writing lever (Schild, 1947). Bathing fluid (Krebs bicarbonate), pH 7·4 of the following composition was used, g/litre (mM): NaCl 6·9 (118); KCl 0·35 (4·7); $CaCl_2 \cdot 2H_2O$ 0·36 (2·5); $MgSO_4 \cdot 7H_2O$ 0·29 (1·17); KH_2PO_4 0·16 (1·2); $NaHCO_3$ 2·1 (25); glucose 1·0 (5·6). A rapid stream of 5% CO_2 in O_2 was used to ensure good stirring. Whenever the preparation was left unattended to relax and stabilize, the bathing fluid was allowed to flow through the bath at approximately 1 ml/min; during the washing phase of the assay cycle, the flow was increased to 10–12 ml/minute. The last dilutions of the drugs were always made in Krebs solution and added to the bath in volumes not exceeding 0·25 ml.

Dilutions of prostaglandin E_2 differing by 10% and 20% in concentration were tested in a random manner on strips under the above experimental conditions to test the capacity of the preparation to discriminate between doses. The final concentrations in the bathing fluid were 3·2 ng/ml (A), 3·6 ng/ml (B) and 4·0 ng/ml (C). Student's *t* test was used for paired comparison; the 1% probability level was the criterion for significance.

Freshly diluted prostaglandin E_2 solutions differing by 20% in concentration (S/U=1·25) were also assayed using a 4-point design (Finney, 1964). The doses were added to the organ bath and randomized in a Latin Square. The methods of calculation and analysis of variance were performed according to Schild (1942).

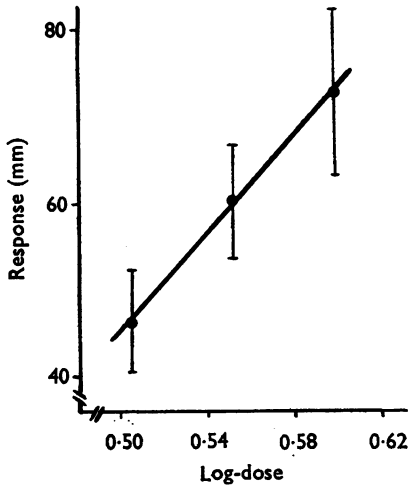
Results

The hamster stomach strip responded quickly even to small doses of prostaglandins E (1–4 ng/ml). The contraction started 2–5 s after the agonist was added to the bath, reached a maximum in 15–25 s and relaxed somewhat spontaneously. If the preparation was adequately loaded (0·5–2·0 g), washing at the moment relaxation commenced caused complete return to the base-line in 2–2·5 minutes. In no instances were there troublesome spontaneous contractions or base line instability which are frequently observed with the rat stomach preparation. If sufficient time was initially allowed for complete relaxation, a steady base line was sustained for a long time.

Only in one of eight preparations was a little tachyphylaxis seen which disappeared either by lengthening the cycle or by intercalating a 5 min recovery period between each set of four doses. This particular preparation was used again, after being kept overnight at 4° C in a moist chamber. The tissue contracted regularly during several hours, suggesting that the storage improved the regularity of the responses. Other preparations have been used quite well during 8 days of intermittent storage at 4° C, before losing the excitability to drugs. Autolytic changes certainly destroyed nervous fibres present in the preparation but did not

affect prostaglandin receptors, so the responses were much more uniform after this treatment.

As can be seen in Fig. 1, the preparation discriminated significantly between doses of prostaglandin E_2 differing by 10%. There was a linear regression of response on log concentration. Though important whatever the bioassay design, this high discriminating power will be particularly relevant if one intends to use the preparation in a 3-point design (assay by bracketing).



Dose		Response
ng/ml	log	(Mean±S.D. (*n*))
A—3·2	0·505	46·0±5·78(6)*
B—3·6	0·556	60·3±6·46(6)*
C—4·0	0·602	72·2±9·85(6)

*$P<0·01$ (From C)

FIG. 1. Discriminating capacity of the hamster stomach strip to different concentrations of prostaglandin E_2.

The results of six bioassays using a 4-point design are summarized in Table 1. A typical tracing is shown in Figure 2. In all cases, the 'unknown' was significantly different from the 'standard' and the regressions significantly different from zero ($P<0·01$). There were no significant differences between the variances of the groups. The regression lines showed no significant deviations from parallelism ($P>0·05$). The mean value of the index of precision λ was low (0·057) reflecting the favourable influence of steep regression lines.

Regarding the accuracy of the assay, only one in six estimates of potency (assay 5) had fiducial limits ($P=0·05$) not bracketing the theoretical value. Though one of the most precise, this particular assay had a rather high percentage error.

In a limited number of experiments, the hamster stomach preparation showed the following sensitivity to different prostaglandins: $E_2/E_1 \sim 1$; $E_2/F_{1\alpha} \sim 65$; $E_2/A_1 \sim 400$.

TABLE 1. *Results of six 4-point assays of prostaglandin E_2 on the hamster stomach* in vitro.

Assay	n	Potency R_1	Potency R	Potency R_2	S.D.	Slope	λ	E %
1	24	1·78	1·42	1·06	12·10	106	0·11	13·6
2	16	1·35	1·21	1·07	3·88	90	0·04	3·2
3	24	1·37	1·26	1·16	5·66	132	0·04	0·8
4	20	1·71	1·38	1·05	7·00	73	0·09	10·4
5	16	1·64	1·49	1·34	4·50	133	0·03	19·2
6	24	1·35	1·25	1·15	5·48	167	0·03	0

R is the estimated potency (theoretical value=1·25) and R_1 and R_2 the P=0·05 fiducial limits. E (%) is the percentage error of the assay and λ is the index of precision.

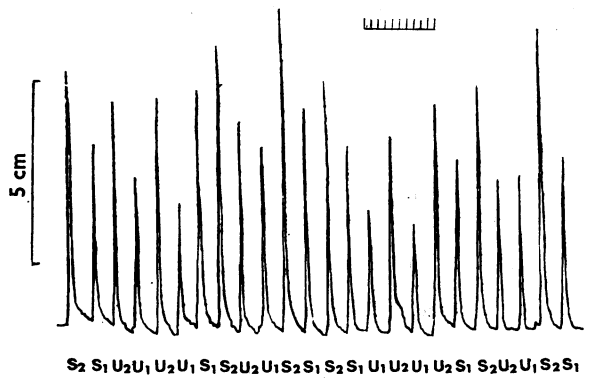


FIG. 2. Typical record of the contractions produced by prostaglandin E_2 on the hamster stomach *in vitro*. Time in minutes. S=standard, U=unknown. Figure retouched.

One of the most distinguishing features of the new preparation was the relative insensitivity to 5-hydroxytryptamine and histamine. Some preparations did not contract even in the presence of 20 μg/ml 5-hydroxytryptamine or 0·2 μg/ml histamine, but did so after the addition of 1 ng/ml prostaglandin E_2 or 1 ng/ml acetylcholine. In spite of the small number of preparations tested, the above figures suggest that the hamster stomach can be used to differentiate between 5-hydroxytryptamine and prostaglandins and between prostaglandins E and F.

Discussion

The new preparation compares favourably with the rat stomach as an assay preparation for prostaglandins. Its dose-response curve is steeper (see slope values in Table 1), its discriminating capacity to different doses of agonist is higher, its responses are faster and relaxation under small loads is easier. These features adequately suit a bracketing assay design and permit the use of even more reliable assay designs. Then, using a 4-point assay for prostaglandin E_2, the index of precision was uniformly low ($\bar{\lambda}$=0·057), much better than the figures reported

for prostaglandin E_1 by Weeks, Schultz & Brown (1968) for the rat stomach (0·165) and gerbil colon (0·115).

The hamster stomach preparation is less sensitive than the rat preparation towards prostaglandins, especially prostaglandin $F_{1\alpha}$ (and probably to $F_{2\alpha}$), but advantage can be taken of this fact in estimations of mixtures of prostaglandins of both series.

The relative insensitivity of the preparation to 5-hydroxytryptamine and histamine renders the hamster stomach suitable for the assay of prostaglandins in body fluids and crude tissue extracts.

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