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A sensitive biological assay for prostaglandin E and acetylcholine

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Superfused hamster stomach strip has been used in the bioassay of prostaglandin E_2 (PGE_2) and acetylcholine. The preparation proved very stable and had no spontaneous movements. The sensitivity to PGE_2 (and PGE_1) was in the range of 10^{-9} g ml^{-1} and to acetylcholine 10^{-12} g ml^{-1}. After treatment with physostigmine the sensitivity to acetylcholine was 10^{-15} g ml^{-1}. In acetylcholine determination the preparation is more sensitive than any other method.

There is a need for reliable bioassay methods for the determination of prostaglandins (PG). There are gas chromatography-mass spectrometry methods and radioimmunoassays but the equipment for the former is very expensive and the latter are still under development. Ubatuba (1973) has described hamster stomach strip (HSS) in organ bath as a sensitive and stable preparation for the estimation of PGE. He found it in many respects better than the widely used rat stomach strip (Vane, 1957). We have used the HSS both in the organ bath and when superfused. The superfusion technique has proved to be even more sensitive and practical than the original method, and it is suitable for biological standardization.

There has also been a lack of a sensitive and reliable technique for the estimation of acetylcholine. Superfused HSS proved to be extremely sensitive to acetylcholine, implying possibilities to be used in the determination of very small amounts of acetylcholine.

MATERIALS AND METHODS

The stomach pouches of adult golden hamsters of either sex (100–150 g) were used. The tissue was cut longitudinally into about 7 cm long strips as described by Ubatuba (1973). The strip was superfused by Krebs bicarbonate solution (Vane, 1957). The contractions were registered either with an isotonic smooth muscle transducer, (Harvard Apparatus, Illinois, No 350/480) or mechanically on a kymograph. The Krebs solution was aerated with 5% CO_2 in oxygen. When biological extracts were tested, atropine (0·1 μg ml^{-1}), propranolol (2 μg ml^{-1}), phenoxybenzamine (0·1 μg ml^{-1}), methysergide (0·2 μg ml^{-1}), and chlorpheniramine (0·1 μg ml^{-1}) were added to the superfusate. The temperature of the superfusate was kept at 37° and the flow rate was 10 ml min^{-1}. The preparation was allowed to stabilize 1–1·5 h before starting the experiments. The drugs (0·1–0·2 ml) were injected into the superfusion cannula of 1 ml volume.

The following drugs were used: PGE_1, PGE_2, $PGF_{2\alpha}$ (Chinoin and Upjohn), 5-hydroxytryptamine-creatinine sulphate and (−)-noradrenaline-(+)-hydrogentartrate (Fluka AG), physostigmine cryst. and histamine dihydrochloride (E. Merck AG), acetylcholine chloride, and arachidonic acid (Sigma), phenoxybenzamine (Alfred

Benzon A/S), propranolol hydrochloride (ICI), atropine sulphate (commercial product, Ph. Nord.), chlorpheniramine (Schering), methysergide (Sandoz AG), acetylsalicylic acid (Rhône-Poulenc), tolfenamic acid (A/S GEA), indomethacin (ICFI). The concentrations refer to the corresponding salts.

RESULTS AND DISCUSSION

General characteristics of the preparation

Our experiments confirmed the good quality of HSS described by Ubatuba (1973). The tissue is easy to prepare, relatively insensitive to environmental disturbances, and keeps its good qualities for 24 h when stored at 4°. The superfusion technique has additional advantages compared with the organ bath system. The superfused HSS is more sensitive, and can be used in cascade. During the bioassay periods we never encountered spontaneous activity. The sensitivity of superfused HSS to various agents is shown in Table 1. When compared with the rat stomach strip (Vane, 1964),

Table 1. *Threshold sensitivities of superfused HSS to various substances.*

Substance		Threshold dose
PGE_1		1 ng ml^{-1}
PGE_2		1
$PGF_{2\alpha}$		10
Arachidonic acid		1000
Noradrenaline		1000
5-Hydroxytryptamine		5000
Histamine		100
Acetylcholine		0·5 pg ml^{-1}
Acetylcholine with physostigmine, (as a single injection before acetylcholine)	0·1 μg	0·1
	1·0 μg	0·01
	10·0 μg	0·5 fg ml^{-1}

the sensitivity of superfused HSS to 5-HT is 1/1000. HSS is slightly less sensitive to PGE_2, 50 times more sensitive to histamine, and 100 000 times more sensitive to acetylcholine (without physostigmine). Although somewhat less sensitive to absolute threshold doses of PGE, the HSS has the advantage of differentiating better between the PGs E and F. In rat stomach strip the relative specificity $PGE_2/PGF_{2\alpha}$ is within the range of 2–3 (Horton, 1972). In HSS the corresponding E/F ratio is about 65 for $PGF_{1\alpha}$ (Ubatuba, 1973) and 10 for $PGF_{2\alpha}$ in the present study (Fig. 1). The steepness of the slope for PGE_2 means a good discriminating ability, and concentration differences of 5% can be measured reliably.

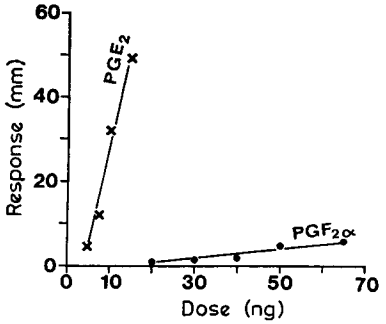

FIG. 1. The dose-response curves of superfused hamster stomach strip to PGE_2 and $PGF_{2\alpha}$. Each point represents mean of 4 values.

The preparation is remarkably sensitive to acetylcholine, concentrations below 10^{-12} g ml^{-1} of a standard solution could be detected. When physostigmine was added, the sensitivity increased in a dose-dependent manner and reached 10^{-15} g ml^{-1} with a single dose of 10 μg of physostigmine. Fig. 2 shows the dose response curves

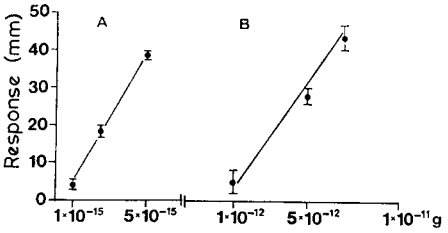

FIG. 2. The dose-response curves of superfused hamster stomach strip (HSS) to acetylcholine, A. 100 ng ml^{-1} of physostigmine in Krebs solution during the preparation of HSS. B. No physostigmine treatment. Each point represents mean of 4 values ± s.e.

of HSS to acetylcholine, and Fig. 3 the increase in sensitivity after physostigmine. The threshold of HSS to acetylcholine is lower than in any other biological preparation. The sensitivity of guinea-pig ileum is 10^{-8} g ml^{-1} and of leech dorsal muscle 2×10^{-11} g ml^{-1} (when treated with physostigmine) (Perry, 1970). Even the best non-biological methods can reach only 5×10^{-13} g ml^{-1} (Cooper, Bloom & Roth, 1974; Goldberg & McCaman, 1973; Jenden, 1972). The superfused HSS has also the advantage of having a rapid time cycle. For leech dorsal muscle the cycle in acetylcholine determination is 10–20 min, but we have found 2–3 min to be suitable for HSS.

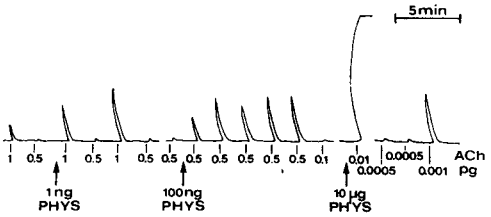

FIG. 3. Potentiation of the effect of acetylcholine (ACh) by physostigmine (PHYS) in hamster stomach strip.

Application in PGE-determination

In our laboratory we have used superfused HSS as a standard method for assaying the biosynthesis of PGE_2. We have compared the inhibitory action of acetylsalicylic acid, indomethacin and tolfenamic acid on the formation of PGE_2 in rabbit kidney medulla PG synthetase (lyophilized microsomal powder). Fig. 4 illustrates the suitability of the superfused HSS for this kind of work.

Application in acetylcholine determination

The remarkable sensitivity of HSS to acetylcholine makes it possible to detect very small amounts of this substance, e.g. in tissue extracts. As a demonstrative application we made a four-point assay at 10^{-15} g ml^{-1} concentration. The HSS was prepared in Krebs solution containing 100 ng ml^{-1} of physostigmine in order to sensitize the preparation. The complete bioassay is seen in Fig. 5. The estimated potency for the test was 80·3% of the standard (theoretical value 80%), and the confidence limits fulfilled the requirements of Pharmacopoea Nordica Editio Fennica Vol. IV.

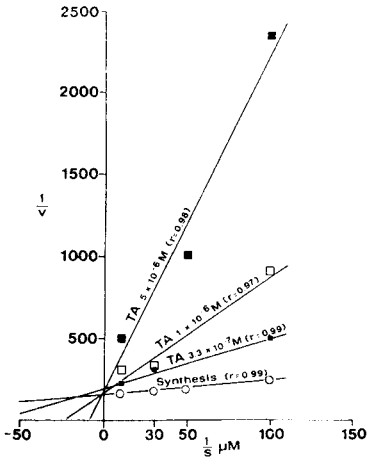

FIG. 4. Conversion of arachidonic acid into PGE_2 in rabbit kidney medulla PG-synthetase system in the presence of glutathione (100 μg) and hydroquinone (20 μg) as cofactors, and tolfenamic acid (TA) as an inhibitor. S = substrate concentration (μM); v = rate of conversion (μM mg^{-1} protein 20 min^{-1}).

FIG. 5. Complete four-point assay of two acetylcholine solutions by using superfused hamster stomach strip. $St_1 = 0{\cdot}75 \times 10^{-15}$ g ml^{-1}, $St_2 = 1{\cdot}5 \times 10^{-15}$ g ml^{-1}, $T_1 = 0{\cdot}6 \times 10^{-15}$ g ml^{-1} and $T_2 = 1{\cdot}2 \times 10^{-15}$ g ml^{-1}.

The preparation is further sensitizable to acetylcholine as a result of continuous superfusion with 100 ng ml^{-1} of physostigmine when a progressive increase in sensitivity is obtained.

In conclusion, we have demonstrated the suitability of superfused HSS in the determination of PGE in amounts as small as 10^{-9} g ml^{-1} and acetylcholine 10^{-15} g ml^{-1}.

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