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Hamster stomach as an isolated preparation for bradykinin assays‡

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The guinea-pig isolated gut has proved most useful for the biological assay of bradykinin to which the preparation is sensitive at 1 ng ml^{-1}, but the tissue's reactivity to acetylcholine, histamine and 5-hydroxytryptamine, led to a search for preparations less sensitive to the other agonists. Rat uterus, stomach and duodenum, made insensitive to acetylcholine by atropine added to the bath, have a high sensitivity to bradykinin and a low sensitivity to histamine. However, rat uterus sometimes shows spontaneous activity. Other preparations from animals have been suggested (Erdos 1970).

Ubatuba (1973) has shown that hamster stomach strips in vitro are useful for the assay of E and F prostaglandins in the nanogram range, with very low sensitivity to histamine and 5-hydroxytryptamine. This has been confirmed by Vapaatalo et al (1976). We have found the preparation to be useful for assay of bradykinin and now we report our results.

Stomach of golden hamsters were removed and cut according to the technique described by Ubatuba (1973). The resulting strips were maintained in Krebs solution in a 5 or 10 ml bath, at 37 °C, gassed by oxygen. After a preparation had been set up, at least 1 h was allowed for equilibration. The contractions of the strips were recorded either by a isotonic frontal lever, stressed by a counter weight of 1g and 10 fold magnification or by a E & M Physiograph, the upper end of the strips being connected to a force displacement transducer.

After addition of bradykinin, the contraction reached a final point at 1 min and relaxation was obtained by washing the preparation, which was left relaxed for 5 min to stabilize before a new cycle was begun by adding another dose of drug to the bath. Not less than three preparations were used for each assay. Sensitivity and dose-response curves were recorded using a synthetic bradykinin solution as a standard. The preparation showed high stability at the base line. Contractions were recorded when 1 or 2 ng ml^{-1} of bradykinin were added to the bath and, sometimes, even with lower a dose. The dose-response curves showed the linear relationship between the log-dose and the height of the responses of the stomach muscle.

Increased sensitivity to some agonists has been reported when other isolated perfused preparations were previously treated with trypsin or other proteases. In the ileum of guinea-pig or uterus of the rat, addition of chymotrypsin (10–50 μg ml^{-1}), chymotrypsinogen or trypsin sensitized the preparations to bradykinin, leaving the responses to histamine, acetylcholine and 5-hydroxytryptamine the same size as before chymotrypsin treatment (Edery 1964, 1968). With the hamster stomach preparation, we found that α-chymotrypsin (Sigma Chem. Comp. bovine pancreas, 3X crystal, lyophyl., salt free), within the range 2·5 to 25 μg ml^{-1}, potentiated the responses to bradykinin, the tissue becoming 5 to 10 times more sensitive to the agonist. Higher doses of α-chymotrypsin may damage the preparations. The α-chymotrypsin was left in contact with the stomach strip for 10 min and, after the preparation was washed, the dose of α-chymotrypsin was repeated to improve the sensitization which may be maintained for 60 min, or more, without further addition of the enzyme.

Assays using Prostaglandins E_1 and F_2 showed that chymotrypsin added to the bath at the same doses that potentiate bradykinin, left the contraction of the muscle at the same size as before the α-chymotrypsin treatment; a similar result has been observed in the guinea-pig ileum or rat uterus for other agonists (Edery 1964).

This relative specificity of α-chymotrypsin to potentiate the hamster stomach to bradykinin, together with its low sensitivity to histamine and 5-hydroxytryptamine, makes the preparation particularly suitable for bradykinin assays.

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