Biochemical Pharmacology, Vol. 26, pp. 2083-2085. Pergamon Press, 1977. Printed in Great Britain.

DIRECT STIMULATORY ACTION OF PENTAGASTRIN ON THE ADENYLATE CYCLASE OF RAT STOMACH MUCOSA

J. NÁFRÁDY and M. WOLLEMANN

First Department of Medicine, University Medical School, 6701-Szeged, P.O.B. 469, Hungary and
Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, 6701-Szeged, P.O.B.521, Hungary

(Received 2 June 1977 ; accepted 6 June 1977)

It was first shown in amphibian gastric mucosa (frog /1/ and mud-puppy /2/)that histamine, gastrin and pentagastrin increase adenylate cyclase activity. The effect of histamine stimulation of adenlyte cyclase activity was also proven in rodent gastric mucosa /3/, however, the stimulating effect of gastrin on the c-AMP levels of gastric mucosa was not demonstrated in vivo until recently. Lewin et. al. /4/ showed a parallel between gastrin binding and stimulation of adenylate cyclase in subcellular fractions of rat gastric mucosa.

The stimulating action of gastrin on adenylate cyclase has been explained until now by Salganik and coworkers /5/ as an indirect effect acting through histamine induction of histidine decarboxylase.

We succeeded in demonstrating in vitro a stimulating effect of low doses pentagastrins on adenylate cyclase activity. As it is not inhibited by metiamide, a known H_2 receptor blocker, we suppose that pentagastrin receptors are directly connected with adenylate cyclase in rat stomach mucosa.

Material and methods

Preparation of particulate cell fractions

Wistar female rats weighing 200-250 g-s were killed by decapitation in ether narcosis. Stomach was excised and washed with 0.05 M pH 7.4 Tris-HCl buffer. The mucosa was scraped off with a blunt knife. The scrape of the mucosa was homogenized in 10 vols of the above buffer at 4 ^{o}C in a Potter Elvehjem homogenizer. (2000 rev/min 3-4 strokes). The homogenate was centrifuged 20 mins at 15.000 rpm at 0 ^{o}C. The supernatant was discarded and the pellet was washed twice with the Tris buffer and the final pellet was suspended in 9 vols of buffer solution.

Adenylate cyclase assay

The reaction mixture contained 50 mM Tris ph 7.4 buffer, 1 mM tetrahydropapaverine, 2 mM ATP (1 µCi ^{3}H-ATP spec. act. 26 Ci/mM or 0.5 µCi 8-^{14}C ATP 1.04 mCi/mM), 2 mM c-AMP, 2 mM $MgCl_2$, 6 mg/ml albumine, 1 mM phosphoenolpyruvate, 0.1 mg pyruvate kinase and 100 µl particulate cell fraction containing 200 µg protein. Incubation was carried out in a final volume of 300 µl at 37 °C for 1,2,3 and 5 mins. The reaction was stopped by boiling the samples at 100 °C for 3 mins in the presence of a 100 µl recovery mixture (10 mM ATP and 1 mM c-AMP) or by the addition of 200 µl of equal parts of 5 % $ZnSO_4$ and 5N $Ba/OH/_2$. 50 µl of the supernatants was applied to Whatmann No. 3. MM for paper chromatography as described previously /8/. Protein was determined according to Lowry et al. /6/. Enzyme activity was linear within the investigated protein concentration and incubation time. All fine chemicals were from Sigma St.Louis , labelled compounds were from New England Corp. Boston. Pentagastrin was a product of ICI, UK. Metiamide was a gift from Smith Kline and French La. Ltd, England.

Results and discussion

The basal adenylate cyclase activity of rat gastric mucosa particulate cell ractions was significantly enhanced by the in vitro addition of histamine or pentagastrin.

Table 1

Dose response relationship of pentagastrin on the basal adenylate cyclase activity of rat gastric mucosa cell free preparations.

	pmoles c-AMP/mg protein/min
Basal Activity	250 ±29 n = 3
Pentagastrin 330 pM	320 ±27 n = 3
" 3.3 nM	365 ±23 n = 3
" 6.6 nM	457 ±21 n = 3
" 33 nM	549 ±46 n = 4

Table 1 shows that the dose curve relationship of pentagastrin revealed maximal effect at 33 nM but doses as low as 330 pM were already causing stimulation. Metiamide, a known H_2 receptor blocker, completely inhibited histamine activation of adenylate cyclase in equal amounts to the applied histamine concentration but displayed no effect on pentagastrin stimulation of enzyme activity (Table 2.).

Table 2

In vitro activating effect of pentagastrin and histamine on the basal adenylate cyclase activity of rat gastric mucosa particulate cell free preparations and the inhibitory effect of metiamide on the stimulation.

	pmoles c-AMP/mg prot/min	
Basal Activity	270 ± 21	n = 6
Histamine 10^{-4}M	458 ± 31	n = 4
Pentagastrin 33 nM	543 ± 46	n = 4
Metiamide 10^{-4}M	274 ± 63	n = 4
Pentagastrin 33 nM + Metiamide 10^{-4} M	531 ± 39	n = 3
Histamine 10^{-4}M + Metiamide 10^{-4}M	277 ± 17	n = 3

The amount of pentagastrin concentration correlated with the activating effect of adenylate cyclase. Maximal effects were achieved with 33 nM above that a decline is observed.

In conclusion pentagastrin acts directly by activating adenylate cyclase of gastric mucosa. In vivo experiments show metiamide inhibition of pentagastrin induced c-AMP elevation which might result in a more long-lasting effect through histidine decarboxylase induction /5,7/ but the immediate effect of pentagastrin (within 5 minutes) is a direct non histamine mediated action.

ACKNOWLEDGEMENTS

The authors wish to thank Prof. Vince Varró for his helpful advice and Mrs. Katalin Horváth for her excellent technical assistance.

References

1. H. Rosen., J.G. Chandler., and F. Multer, Surg. Forum 221, 291 /1971/.
2. S. Nakajima., B.I. Hirschowitz, and B. Sachs, Arch. Biochem. Biophys. 143, 123 /1971/.
3. P.B. Ramwell, and J.E. Shaw, J. Physiol 195, 34P /1968/.
4. M. Lewin., A. Soumarmon, J.P. Bali, S, Bonfils, J.P. Girma, J.L. Morgat, and P. Fromageot, FEBS Letters 66, 169 /1976/.
5. R.I. Salganik, S.V. Argutinskaja, and R.I. Bersimbaev, Experientia 28,1190 /1972/.
6. O.H. Lowry, N.J.Rosenbrough, A.Farr, and R.J.Randall,J.Biol.Chem.193, 265 /1951/.
7. K.T. Bunce, M.E.Parsons, and Rollings, A.Nita,Br.J.Pharmacol.58, 149 /1976/.
8. M. Wollemann, Adv. in Biochem. Psychopharmacol. 9, 731 /1974/.