Effect of the H_1-Histamine-Like Agonist Material Extracted from Bovine Spleen on 3': 5'-Cyclic Nucleotides Content in Guinea-pig Ileal Smooth Muscle

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The effect of a water-soluble «histamine-free» splenic material that mimics the H_1-receptor mediated contractile action of histamine on the guinea-pig ileum has been studied upon concentrations of cGMP and cAMP in slices of this smooth muscle preparation.

The splenic extract induced a rapid and sustained decrease in the concentration of cGMP accompanied by a slow decrease in cAMP content in the ileal tissue.

These results indicate that the smooth muscle-stimulating agent in splenic extract had no increasing effect on cGMP content as could be expected from the hypothesis that cGMP and cAMP might mediate the smooth muscle contraction and relaxation respectively.

The data are not compatible with the general hypothesis that the action of histamine and histamine-like agonists on H_1-receptors is associated with an increased concentration of cGMP.

Key words: «Histamine-free» splenic extract. Guinea-pig ileal smooth muscle, cAMP, cGMP.

Since GANDARIAS (11) obtained a water soluble material extracted from spleen, lung and pancreas of rats and rabbits with striking activity on smooth muscle, there has been a continued interest in this smooth muscle-active agent. Further experiments have demonstrated a similar activity on smooth muscle with extracts of bovine spleen (1, 2, 13, 16) and rabbit renal cortex (7). Several studies carried out to set up the mechanism of the action and the nature of this active factor have discarded the possibility of involvement of adrenergic, cholinergic and tryptaminergic mechanisms as mediators in various smooth muscle preparations (1, 2, 12, 13, 16, 19). These studies have also discarded the possibility of this smooth muscle-active factor being a prosta-

glandin, renin, angiotensin, catecholamine, 5-hydroxy-tryptamine, histamine, bradykinin, a nucleotide, a small organic product of local metabolism or an ion (1, 2, 7, 12, 13, 16). Recent experiments suggest the involvement of histaminergic mechanisms in the activity displayed by this active factor in the intestinal smooth muscle (14, 16). It has been stated that the H_1 histamine receptors may be involved in the contractile response of the guinea-pig ileal smooth muscle brought about by «histamine-free» water-soluble extracts of bovine spleen (2). Taking this into account and the various descriptions referring specific modifications on cyclic GMP and cyclic AMP tissue levels when histamine or histamine-like agonists interact with H_1 and H_2 receptors (5, 6, 9, 10, 17, 20, 22, 23, 30) research has been done on the effect of the H_1 histamine-like agonist material present in the bovine splenic extracts on cyclic AMP and cyclic GMP levels in guinea-pig ileum. The data indicate that the modifications in the amount of cyclic AMP and cyclic GMP brought about by the splenic extract are not attributable to its interaction with H_1 receptors in the ileal smooth muscle tissue.

Materials and Methods

Preparation of splenic extracts. Extracts were obtained from fresh bovine spleen. The raw material was bruised and salted out by addition of 5 ml of 0.3N NaOH and 5 ml 5 % $ZnSO_4$ solution (4) per g of wet tissue. The material was then homogenized in a MSE ultrasonic homogenizer (6 kHz × 3 min) and centrifuged at 5200 *g* for 5 min. The precipitate was discarded and the supernatant collected and stored at 2-4° C. Under these conditions the extract remained active for at least one week. When lyophilized, the activity remained unchanged for several months.

Preparation and incubation of ileal slices. Guinea-pigs of either sex weighing about 400 g were stunned by a blow on the back of the head and then exsanguinated. Their ilea were quickly dissected and washed with Tyrode's solution at 37° C. The Tyrode solution had the following composition (g/l): NaCl 8, KCl 0.2, $CaCl_2 \cdot H_2O$ 0.18, $MgSO_4 \cdot 7H_2O$ 0.26, $NaH_2PO_4 \cdot 2H_2O$ 0.013, $NaHCO_3$ 1.0, glucose 1.0 and atropine sulphate 0.002. Further manipulations of the ilea were carried out following the method described by LEE *et al.* (27). The tissue slices obtained were suspended in Tyrode's solution warmed to 37° C. Aliquots (2.5 ml) of the tissue suspension containing about 27 mg of protein, measured by the method of LOWRY *et al.* (28), were transferred directly into glass homogenizers and incubated in Tyrode's solution at 37° C for various periods in the presence of splenic extract (SE) in doses of 0.1 ml, equivalent to 10 mg fresh tissue. The muscularis tissue suspension samples were inactivated by boiling (15). Tissue samples were immediately homogenized and the precipitates were removed by centrifugation. The cyclic nucleotides in the supernatant were estimated by RIA using commercial kits «Radiochemical Centre» (15). The recoveries of cyclic AMP and cyclic GMP were evaluated by adding 16 pmol per 50 μl of cAMP to several muscularis tissue suspension aliquots and 4 pmol per 100 μl of cGMP to others. These samples were subjected to the general procedure above described.

Statistical evaluation. The results are expressed as means ± s.e. mean for the number of experiments indicated (n). Significant differences between means were calculated by Student *t*-test.

Results

The effect of incubation in the presence of SE (0.1 ml corresponding to 10 mg fresh tissue) on cyclic AMP and cyclic GMP content in muscularis slices from guinea-pig ileum is shown in figure 1. The SE caused a significant decrease in cyclic AMP content at 40 s of incubation. This response was apparent within 10 s, the earliest time studied. The decrease observed was not sufficiently rapid to explain a possible involvement of cyclic AMP in the previously reported (2) ileal smooth muscle contraction induced by SE.

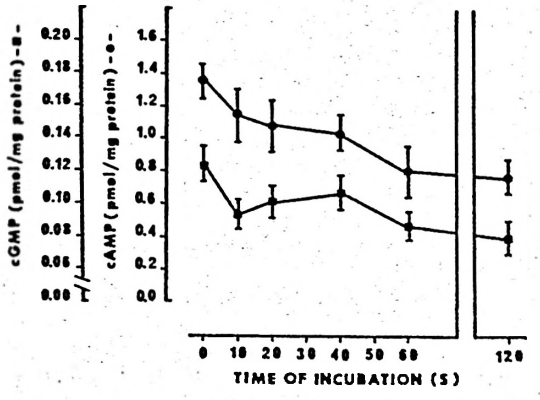
The experiments performed to evaluate the effect of SE on cyclic GMP showed that the SE caused a rapid and significant decrease in cyclic GMP content within 10 s. Afterwards the endogenous concentration of cyclic GMP remained lowered with respect to control value. However, at 20 and 40 s of incubation a slight increase in cyclic GMP content was observed. These values were not significantly different from those of control.

Since this slight increase was slow and not significant, only the rapid decrease observed was correlated with a possible role of cyclic GMP in the smooth muscle contractile response to SE.

Discussion

There are a considerable number of evidences that support the presence of a water soluble smooth muscle active material in various tissue extracts from several species (1, 2, 7, 11-14, 16, 19).

In 1973 GOLDBERG *et al.* (17) proposed the «yin-yang» hypothesis. This hypothesis considers that the cyclic AMP and cyclic GMP are the opposing arms of a bidirectional intracellular control system. It has found support in studies on several biological systems including experiments on the spontaneous and drug-induced relaxation and con-



Time of incubation (s)	cAMP (pmol/mg protein)	n	cGMP (pmol/mg protein)	n
0*	1.380±0.09	16	0.125±0.009	23
10	1.140±0.14	9	0.094±0.007[a]	19
20	1.081±0.15	16	0.102±0.009	17
40	1.045±0.11[a]	9	0.108±0.01	17
60	0.808±0.14[b]	11	0.087±0.007[b]	20
120	0.762±0.10[b]	9	0.080±0.009[b]	15

Fig. 1. *Effect of splenic extract on cAMP and cGMP content in muscularis slices from guinea-pig ileum as a function of incubation time.*

Muscularis slices from guinea-pig ileum were incubated with splenic extract (0.1 ml corresponding to 10 mg fresh tissue) for various times, as indicated. Each value represents the mean ± s.e.m. of assays on six tissue samples, each of which was analyzed in duplicate. The table shows the cAMP and cGMP concentration values at each time of incubation and their statistical significances respect the zero time control. (* mean values in absence of S.E. a: $p < 0.05$, b: $p < 0.01$)

traction of smooth muscle preparations (5, 17, 24, 25, 27). In addition, there are several works that describe increases on cyclic GMP in response to histamine and histamine-like agonists mediated by H_1 receptors (5, 6, 18, 23, 30).

This H_1 mediated effect also induces a decrease on cyclic AMP content in the intestinal smooth muscle, presumably via stimulation of phosphodiesterase (23).

Based on these previous reports, an association between H_1 mediated contractile effect displayed by splenic extracts (2) and changes on cyclic nucleotides content in the ileal smooth muscle tissue similar to those proposed by the «yin-yang» hypothesis could be expected.

From the present results the effect of the splenic extracts on cAMP has similarities to those previously described by other authors (3, 23), who have described decreases on cyclic AMP levels in intestinal smooth muscle during the contractile response induced by histamine. Conversely, the effect of splenic extracts on cyclic GMP was clearly different from those previously described indicating increases in cyclic GMP associated with the contractile response of the intestinal smooth muscle induced by histamine and several other smooth muscle-stimulating agents such as cholinergic drugs (5, 27). There are studies describing similar discrepancies between smooth muscle activity and changes in cyclic nucleotides content (8, 26, 29). These studies and the present work provide evidences against the «yin-yang» hypothesis. In addition, an exclusive role of cyclic AMP and cyclic GMP as alternative intracellular regulators in smooth muscle motility has been questioned by HARBON *et al.* (21).

Taking this all into account, the results of the present study suggest that cyclic nucleotides are not directly involved in the intracellular mechanism of action of the H_1-histamine-like agonist material extracted from bovine spleen in guinea-pig ileum.

Resumen

Se estudia el efecto del material esplénico hidrosoluble «libre de histamina» sobre el contenido de GMPc y AMPc en fragmentos musculares de íleon de cobaya.

El extracto esplénico promueve un descenso rápido y sostenido del nivel de GMPc acompañado por un descenso lento del contenido tisular de AMPc.

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