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SHORT COMMUNICATIONS

Comparison of Mg^{2+} and Mn^{2+} as metal cofactors for histamine-stimulated adenylate cyclase in guinea pig gastric mucosa*

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Histamine (HA)-stimulated adenylate cyclase (AC) in the gastric mucosa of various species has been used to evaluate antagonists [1–4] and agonists [5–8] at HA H_2-receptors. The present study was undertaken to compare Mg^{2+} and Mn^{2+} as metal cofactors and to determine requirements for GTP. Mn^{2+} was selected for study because one of our groups† has shown that it supports higher reaction velocities than Mg^{2+} for AC activity present in membranes isolated from ciliary processes. Further, unlike Mg^{2+}, it does not support the inhibitory subunit of the AC complex [9, 10]. The results show that Mn^{2+} was more effective than Mg^{2+} in supporting gastric mucosal HA-stimulated AC activity without altering the apparent affinity of the H_2-receptor for agonists or antagonists.

Methods and materials

Male, Hartley albino strain guinea pigs, 400–800 g, fed *ad lib.* were used in all experiments. The animals were killed by a blow on the head and exsanguinated. The stomach was rapidly removed, cut open along the lesser curvature, and rinsed with ice-cold saline to remove adhering contents. The fundic mucosa was removed by scraping and was homogenized in 9 vol. of buffer in a Wheaton–Dounce homogenizer (five strokes with pestle B followed by five strokes with pestle A). The composition of the buffer was (mM): sucrose, 250; Tris–HCl (pH 7.5), 20; EDTA, 5; ethyleneglycolbis(amino-ethylether)tetra-acetate (EGTA), 1; and dithiothreitol, 5. Membranes were isolated by centrifugation at 3000 *g* for 10 min. The resulting pellet was washed twice with sucrose-free homogenizing buffer. The final pellet was resuspended by homogenization in 9 vol. of ice-cold sucrose-free homogenizing buffer and kept on ice until use. Membranes were prepared freshly for each experiment.

Adenylate cyclase activity was determined by a modification of the method of Solomon *et al.* [11]. The composition of the reaction mixture was (mM): Tris–HCl (pH 7.5), 20; EDTA, 0.75; EGTA, 0.15; ATP, 0.2; [α-^{32}P]ATP, 2–3 × 10^6 cpm; GTP, 0.01; creatine phosphate, 1.25; creatine phosphokinase, 125 μg; dithiothreitol, 0.25; theophylline, 1; cyclic AMP (cAMP), 1; $MnCl_2$ or $MgCl_2$, as noted; membranes, 50–120 μg protein; and HA or tiotidine, as noted, in a final volume of 0.2 ml. After a 5-min preincubation at 37°, the reaction was initiated by the addition of [α-^{32}P]ATP. Incubations were carried out for 5 min and stopped by the addition of 0.1 ml of a solution containing 2% sodium dodecyl sulfate, 40 mM ATP, and 1 mM cAMP at pH 7.5. [^{3}H]cAMP, about 15,000 cpm, was added to monitor the recovery of cAMP isolated by the double column method of Solomin *et al.* [11]. All assays were performed in triplicate. Rates are expressed as pmol cAMP·mg protein^{-1} · min^{-1}.

The apparent activation constants, K_a, for HA, GTP, and Mg^{2+} were determined by nonlinear regression analysis using the program Allfit [12]. The apparent activation and inhibition constants for free Mn^{2+} were determined by nonlinear regression analysis using the program NLLSQ (CET Research Group, Ltd., Norman, OK). The apparent K_i for tiotidine was calculated using the expression $K_i = IC_{50}/(1 + [A]/K_a)$. The concentrations of free Mn^{2+} and Mg^{2+} were approximated by sequentially solving the quadradic equation using stability constants of the chelators in descending order. The concentration of free metal obtained at each step was used as the concentration of total metal for the next step.

[α-^{32}P]ATP and [^{3}H]cAMP were obtained from ICN (Irvine, CA). ATP (GTP free), GTP, cAMP, phosphocreatine, creatine phosphokinase, theophylline, and HA were obtained from the Sigma Chemical Co. (St. Louis, MO). Titotidine was provided by Dr. David McCurdy, Stuart Pharmaceuticals Division of ICI Americas (Willmington, DE). The pH of all reagent stock solutions was adjusted to 7.4 with Tris base to avoid pH artifacts [13]. BioRad (Richmond, CA) assay kits were used for protein determinations.

Results and discussion

The effects of Mn^{2+} and Mg^{2+} on HA-stimulated AC are shown in Fig. 1. The results of these studies established that the optimal concentration for total Mn^{2+} was about 2 mM and 10 mM for Mg^{2+} and that Mn^{2+} supported higher reaction rates. Figure 1 alsso shows that Mn^{2+} exhibited biphasic kinetics on HA-stimulated AC, but not on basal activity. In separate experiments (data not shown), the apparent kinetic constants for free Mg^{2+} and free Mn^{2+} were estimated. The apparent K_a and K_i values for Mn^{2+} were 24 ± 1.0 μM and 2.9 ± 1.2 mM (N = 3) respectively.

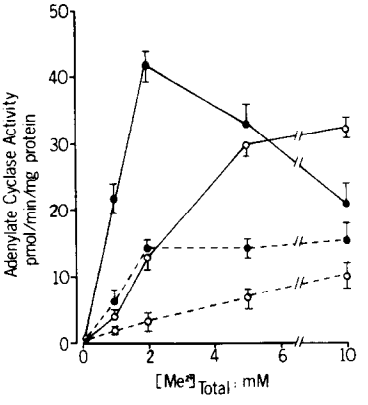

Fig. 1. Effects of varying [Mg^{2+}] (○) and [Mn^{2+}] (●) on HA-stimulated (——) and basal (– – –) AC activity in gastric mucosal membranes. The concentration of histamine was 300 μM. The data are mean ± SE for three experiments. [Me^{2+}] = [cation $^{2+}$].

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The apparent K_a value for Mg^{2+} was 0.98 ± 0.07 mM (N = 3). Similar experiments were carried out to determine the apparent K_a for GTP in the presence of 300 μM HA and optimal concentrations of Mg^{2+} or Mn^{2+}. In the presence of Mg^{2+} or Mn^{2+}, the apparent K_a values for GTP were 0.24 ± 0.04 and 0.79 ± 0.19 μM (N = 3) respectively. The results of these experiments established that optimal rates for HA stimulation could be obtained in the presence of 10 μM GTP and either 2 mM total $MnCl_2$ or 10 mM $MgCl_2$.

The next series of experiments were undertaken to determine if the choice of the divalent cation influenced the apparent affinity of the H_2-receptor for an agonist, HA, or for a competitive antagonist, tiotidine [14, 15]. The results of the experiments with HA are shown in Fig. 2. In the presence of optimal concentrations of GTP and Mg^{2+} or Mn^{2+}, the apparent K_a values for HA were 14.2 ± 4.0 and 14.0 ± 2.7 μM (N = 3) respectively. These values are well within the range, 1 μM [3, 4] to about 30 μM [6], reported by others. The results of experiments with tiotidine are shown in Fig. 3. The IC_{50} values for tiotidine in the presence of Mg^{2+} or Mn^{2+} were 1.3 ± 0.4 and 1.3 ± 0.6 μM (N = 3), respectively, which correspond to a K_i value of 58 nM. This is in fair agreement with the values found in the literature for the antagonism of H_2-receptors: 16–50 nM [14], 22 nM [15], and 24 nM [1].

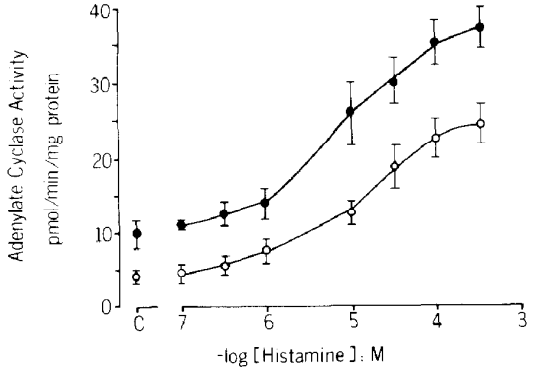

Fig. 2. Comparison of 2 mM total Mn^{2+} (●) and 10 mM total Mg^{2+} (○) on the concentration–response curves for HA-stimulation of gastric mucosal AC. The data are mean ± SE for three experiments, and the lines are lines of best fit. The activity in the absence of added histamine is shown at C on the Y-axis.

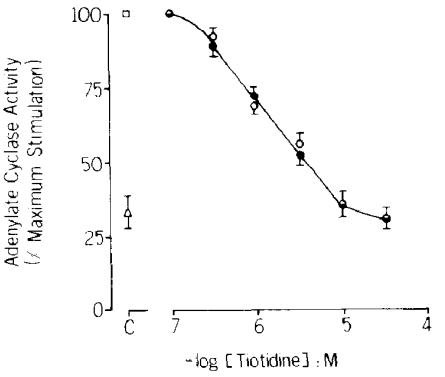

Fig. 3. Comparison of 2 mM total Mn^{2+} (●) and 10 mM total Mg^{2+} (○) on the inhibition of HA-stimulated gastric mucosal AC by tiotidine. The values for basal (no added HA) activity (△) and maximal (300 μM HA) activity (□) are shown at C on the Y-axis. The data are mean ± SE for three experiments. The line is the line of best fit.

The results of the present study show that Mn^{2+} can be used instead of Mg^{2+} in studies on gastric mucosal HA-stimulated AC. Mn^{2+} has the advantage of supporting higher reaction velocities without altering the apparent affinity of the H_2-receptor for antagonists or agonists. In addition, the results of studies in progress suggest that the relative maximal response to histamine H_2-partial agonists is not altered by Mn^{2+}.

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