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Distinct ONE-GC Transduction Modes and Motifs of the Odorants: Uroguanylin and CO₂

Teresa Duda^{*} and Rameshwar K. Sharma^{*}

Research Divisions of Biochemistry and Molecular Biology, The Unit of Regulatory and Molecular Biology, Salus University, Elkins Park, Pennsylvania 19027

Abstract

In a subset of the olfactory sensory neurons ONE-GC\$ membrane guanylate cyclase is a central component of two odorant-dependent cyclic GMP signaling pathways. These odorants are uroguanylin and CO₂. The present study was designed to decipher the biochemical and molecular differences between these two odorant signaling mechanisms. The study shows (1) in contrast to uroguanylin, CO₂ transduction mechanism is Ca²⁺-independent. (2) CO₂ transduction site, like that of uroguanylin-neurocalcin δ , resides in the core catalytic domain, aa 880-1028, of ONE-GC. (3) The site, however, does not overlap the signature neurocalcin δ signal transduction domain, ⁹⁰⁸LSEPIE⁹¹³. Finally, (4) this study negates the prevailing concept that CO₂ uniquely signals ONE-GC activity [Sun, L. et al., (2009) *Proc. Natl. Acad. Sci. USA.* 106, 2041-2046; Guo, D. et al., (2009) *Biochemistry* 48, 4417-4422]. It demonstrates that it also signals the activation of photoreceptor membrane guanylate cyclase ROS-GC1. These results show an additional new transduction mechanism of the membrane guanylate cyclases and broaden our understanding of the molecular mechanisms by which different odorants using a single guanylate cyclase can regulate diverse cyclic GMP signaling pathways.

Keywords

membrane guanylate cyclase; ONE-GC; odorant transduction; bicarbonate; cyclic GMP; signal transduction

Introduction

The odorant signal begins at the ciliated apical border of the olfactory sensory neurons located in the main olfactory epithelium. To be transmitted to the defined cortical neurons of the brain where the final perception of SMELL is completed, in the first step the odorant generates an electrical signal. The biochemical term for this process is odorant-transduction [reviewed in 1-4; Fig. 1 ref 4]. In this process the odorant signal is first converted to its second messenger and, then, the second messenger transforms the signal into an electrical signal. This, then,

^{\$}**Abbreviations:** aa, amino acid; Catd, catalytic domain; ONE-GC, Olfactory Neuroepithelial membrane Guanylate Cyclase; ROS-GC, Rod Outer Segment membrane Guanylate Cyclase; tm-catd, transmembrane linked catalytic domain.

^{*}To whom correspondence should be addressed: phone: 215-780-3124 (RKS), 215-780-3112 (TD) fax: 215-780-3125
rsharma@salus.edu, tduda@salus.edu.

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through action potentials becomes a means of signal transmission and the final perception of SMELL in the cortical layers of the brain.

The major second messenger of the odorant signal is cyclic AMP [5-9]. In the incremental development of the field, beginning in 1995 [10-12], it has now been finally established that cyclic GMP is also the second messenger of the odorant signal [reviewed in: 2-4]. This signaling pathway resides in a small population of the olfactory receptor neurons (ORNs) and is independent of the cyclic AMP signaling pathway [11-12]. The pathway begins with the ONE-GC membrane guanylate cyclase (also named GC-D [10]) which is co-present with the cyclic GMP-dependent components, cyclic GMP-specific cyclic nucleotide-gated channel subunit, CNGB3, and a cyclic GMP-dependent phosphodiesterase, PDE2 [11-12] thus, it appears to be linked with them for its down stream functions.

It is important to understand that the *modi operandi* of these two pathways are radically different. In contrast to the cyclic AMP, cyclic GMP pathway does not function through the GTP-binding protein, G_{olf} . It directly originates from ONE-GC, which is both the receptor for the odorants uroguanylin [13-14] and green pepper [15-16], and also the transducer through its guanylate cyclase activity. Thus, on the lines of the prototype model of ANF-RGC membrane guanylate cyclase signal transduction [17], coexistence of the uroguanylin receptor and guanylate cyclase activities on a single transmembrane spanning polypeptide chain demonstrates that the mechanism of signal transduction involving mediation by the second messenger, cyclic GMP, is different from the adenylate cyclase system. The single polypeptide chain of ONE-GC contains both the information for signal recognition and its translation into a second messenger. This makes the cyclic GMP signal transduction pathway more direct and, theoretically faster.

ONE-GC in addition to being a direct odorant receptor and transducer possesses an additional intriguing feature. Indirectly, through carbonic anhydrase enzyme, it senses atmospheric CO_2 and gets accelerated in its production of cyclic GMP [18-19]. Fragmentary evidence suggests that the direct and the indirect odorant signal transduction mechanisms of ONE-GC are different [18-19]. This presentation investigates this problem and demonstrates the differences at the functional and structural levels.

Materials and Methods

ONE-GC tm-catd mutant

Construction of the mutant is described in [14] and the mutant is schematically presented in figure 1.

ONE-GC Δ^{908} LSEPIE 913 mutant

Deletion of the 908 LSEPIE 913 motif from the ONE-GC sequence was done using Quick Change mutagenesis kit (Stratagene) and mutagenic primers: forward primer 5'-GGCTTTACCACCATCTCAGCCGTGTGGTGGGCTTCCTCAATGAT-3'; reverse primer 5'-ATCATTGAGGAAGCCACCGCTGAGATGGTGGTAAAGCC-3'. The deletion was verified by sequencing. The mutant is schematically presented figure 1.

Expression in COS cells

COS-7 cells were transfected with the wild type (wt) ONE-GC, its tm-catd or Δ^{908} LSEPIE 913 mutants, or ROS-GC1 cDNA through the standard procedures [20]. After sixty hours the cells were washed with 50 mM Tris-HCl (pH 7.5)/10 mM buffer, homogenized, centrifuged at 5000g and washed several times with the same buffer. The resulting pellet represented crude membranes.

Guanylate cyclase activity assay

Membrane fractions of transfected COS cells were assayed for guanylate cyclase activity as described previously [14, 15, 17]. Briefly, membranes were incubated on ice with NaHCO_3 and/or neurocalcin δ in the assay system containing 10 mM theophylline, 15 mM phosphocreatine, 20 μg creatine kinase and 50 mM Tris-HCl, pH 7.5, adjusted to 10 μM free Ca^{2+} or 1 mM EGTA concentrations with pre-calibrated Ca^{2+} /EGTA solutions (Molecular Probes). The total assay volume was 25 μl . The reaction was initiated by addition of the substrate solution (4 mM MgCl_2 and 1 mM GTP, final concentrations) and maintained by incubation at 37 °C for 10 min. The reaction was terminated by the addition of 225 μl of 50 mM sodium acetate buffer, pH 6.2 followed by heating on a boiling water bath for 3 min. The amount of cyclic GMP formed was determined by radioimmunoassay [21].

Results

Bicarbonate stimulates ONE-GC in a Ca^{2+} -independent manner

Bicarbonate, the mediator of the odorant atmospheric CO_2 , stimulates ONE-GC activity [18, 19, 22]. The stimulation is through the intracellular domain of ONE-GC [19, 22]. This situation is similar to the uroguanylin neurocalcin δ -modulated Ca^{2+} -signaling step of the ONE-GC activation, which also occurs at the intracellular domain of ONE-GC [23]. To determine whether the bicarbonate stimulation of ONE-GC requires Ca^{2+} , ONE-GC was expressed in the heterologous COS cell system and their membranes were exposed to the increasing concentrations of sodium bicarbonate in presence of the saturating concentration of Ca^{2+} (10 μM) Ca^{2+} ; the sample without Ca^{2+} , containing 1 mM EGTA, served as control. In both cases with identical patterns, the bicarbonate stimulated ONE-GC activity in a dose-dependent fashion (Fig. 2A). EC_{50} of the bicarbonate was ~20 mM. The ONE-GC activity was elevated from the basal level of ~38 pmol cyclic GMPmin⁻¹mg⁻¹ protein to ~240 pmol cyclic GMPmin⁻¹mg⁻¹ protein. To compare these results with those of the neurocalcin δ -modulated Ca^{2+} signaling step of ONE-GC activation, the Ca^{2+} signaling of ONE-GC was assessed side by side with the bicarbonate. The samples contained saturating amount of Ca^{2+} and increasing concentrations of neurocalcin δ ; the control samples contained no Ca^{2+} . In accordance with the earlier results and conclusions [14, 15, 24], neurocalcin δ -modulated Ca^{2+} signaling of ONE-GC was strictly Ca^{2+} -dependent (Fig. 2B). These results demonstrate that unlike the Ca^{2+} -modulated odorant uroguanylin step, the bicarbonate stimulation of ONE-GC is Ca^{2+} -independent. It is direct and does not involve any accessory factors.

The 6.3-fold bicarbonate-dependent and Ca^{2+} -independent stimulation of ONE-GC shown here (Fig. 2A) is comparable to the earlier reports, one showing ~4.6- [19] and the other, ~3-fold [22]. Similarly the present EC_{50} value for bicarbonate (Fig. 2) is comparable to the other reported values [19].

Bicarbonate- and uroguanylin-modulated domains reside in the core catalytic domain of ONE-GC

The core catalytic domain of ONE-GC extends from the aa M⁸⁸⁰ to P¹⁰²⁸ [14] and the neurocalcin δ -modulated Ca^{2+} signal transduction site resides in this domain, covering its aa M⁸⁸⁰-L⁹²¹ segment [14]. To determine whether the bicarbonate-modulated site also resides in the ONE-GC core catalytic domain, the tm-catd mutant expressed in COS cells was used. It is noted that this mutant represents the core catalytic domain of ONE-GC, which is attached to the transmembrane domain (Fig. 1) for its expression in the membrane fraction of the cell [14]. The mutant and the wt-ONE GC have almost identical basal guanylate cyclase activities (Fig. 1, column [A]), indicating that the tertiary structures of their core catalytic domains are also identical. Bicarbonate stimulated the tm-catd mutant (Fig. 2C). The stimulation was similar to that of the wt-ONE-GC (Compare figures 2A and 2C: maximal stimulation of ~200

pmol cyclic GMP min⁻¹ mg⁻¹ protein achieved at ~40 mM of bicarbonate; in EC₅₀ for bicarbonate 20 mM). As demonstrated previously [14], the mutant is also responsive to the uroguanylin-neurocalcin δ -modulated Ca²⁺ signaling. These results show that, like the Ca²⁺-signaling site, the bicarbonate-modulated ONE-GC signal transduction site resides in the core catalytic domain of ONE-GC.

The two earlier reports have arrived also at the conclusion that the bicarbonate-modulated site resides in the catalytic domain of ONE-GC. However, their description of the catalytic domain is not precise and do not warrant this conclusion. In one report the catalytic domain is represented as the ONE-GC aa 850-1110 segment [19] and in the other, as aa 546-1071 segment [22]. The aa 850-1110 segment besides the catalytic domain contains part of the predicted dimerization domain and the C-terminal extension segment of ONE-GC; the aa 546-1071 segment besides the catalytic domain contains the kinase homology and dimerization domains and part of the C-terminal extension segment.

The bicarbonate and the Ca²⁺-modulated signal transduction sites in ONE-GC are different

The target site of the Ca²⁺-bound neurocalcin δ in ONE-GC extends from M⁸⁸⁰ to L⁹²¹ [14]. The information that the bicarbonate signal transduction mechanism is Ca²⁺-independent suggested that the bicarbonate-modulated domain in ONE-GC should be different from that of neurocalcin δ .

To test this prediction, the cues from the structures of the functional epitopes of the photoreceptor ROS-GC1 were used [25]. Like for ONE-GC, neurocalcin δ is a Ca²⁺-sensor of ROS-GC1. The target site of Ca²⁺-bound neurocalcin δ in ROS-GC1 resides also in the core catalytic domain, G⁸¹⁷-Y⁹⁶⁵ and covers a region V⁸³⁷-L⁸⁵⁸ [25]. The core catalytic domains of ROS-GC1 and ONE-GC are 85% homologous and there is 88% homology between the neurocalcin δ target sites in these two cyclases. Besides these similarities, the cyclases have an additional common structural motif. The motif in ROS-GC1 is ⁸⁴⁵MSEPIE⁸⁵⁰ and in ONE-GC, ⁹⁰⁸LSEPIE⁹¹³. This signaling motif in ROS-GC1 represents the signature domain of the neurocalcin δ function [25]. It was reasoned that this information could be used to discriminate the structural differences between the bicarbonate- and the neurocalcin δ -modulated Ca²⁺-signaling sites in ONE-GC.

To accomplish this task, the first question was: Is ⁹⁰⁸LSEPIE⁹¹³ also a signature domain in neurocalcin δ -modulated Ca²⁺ signaling of ONE-GC?

The answer was YES. The Δ ⁹⁰⁸LSEPIE⁹¹³ mutant of ONE-GC expressed in COS cell membranes lost majority, ~65 %, of its neurocalcin δ -stimulated activity (Fig. 3A). However, its basal activity remained the same. These results demonstrated that the mutation did not affect the tertiary structure of the catalytic domain, yet it eliminated most of its Ca²⁺-dependent regulatory activity. Thus, in ONE-GC the signature motif of the neurocalcin δ modulation is ⁹⁰⁸LSEPIE⁹¹³; barring one residue, it is identical to the corresponding motif in ROS-GC1. Thus, it can be concluded that this motif is the universal code for the neurocalcin δ -Ca²⁺-modulation of any membrane guanylate cyclase.

This conclusion would also suggest that the ⁹⁰⁸LSEPIE⁹¹³-deletion mutant would respond normally to the bicarbonate stimulation. The results show that it does (Fig. 3B). They prove that the bicarbonate and the Ca²⁺-signal transduction sites in ONE-GC are different; they do not overlap. Conclusions of the distinct sites for Ca²⁺ and bicarbonate in ONE-GC were further corroborated by the following experiment.

Membranes of COS cells expressing ONE-GC were exposed simultaneously to 2 μ M neurocalcin δ and 30 mM sodium bicarbonate in the presence of 10 μ M CaCl₂. The

concentrations of neurocalcin δ and the bicarbonate chosen for the experiment were those causing almost maximal activation of ONE-GC. For comparison, side-by-side the membranes were treated with Ca^{2+} -bound neurocalcin δ or bicarbonate alone. Results of these experiments are presented in figure 3C. The ONE-GC activity in the presence of both 2 μM neurocalcin δ and 30 mM bicarbonate was 345 ± 20 pmol cyclic GMP $\text{min}^{-1} \text{mg}^{-1}$ protein. The activity with neurocalcin δ alone was 110 ± 5 pmol cyclic GMP $\text{min}^{-1} \text{mg}^{-1}$ protein and with bicarbonate alone was 210 pmol cyclic GMP $\text{min}^{-1} \text{mg}^{-1}$ protein. These results show that the effects of neurocalcin δ and bicarbonate on ONE-GC activity are additive, and confirm the earlier conclusion that the Ca^{2+} -independent bicarbonate and the Ca^{2+} -dependent uroguanylin-neurocalcin δ transduction pathways are mechanistically separated.

Bicarbonate also signals the activation of ROS-GC1

In view of an extremely close sequence homology between the core catalytic domains and almost total homology between the signature domains of neurocalcin δ -modulated Ca^{2+} signaling domains in ONE-GC and ROS-GC1, it was surprising to note two earlier reports claiming that the bicarbonate signaling of ONE-GC is unique and it does not occur in any other member of the membrane guanylate cyclase family [19–22]. The bases of these conclusions were revisited as follows.

Membranes of COS cells expressing ROS-GC1 were exposed to the increasing concentrations (0 to 80 mM) of sodium bicarbonate and the guanylate cyclase activity was assessed. As control, membranes of COS cells transfected with empty pcDNA3 vector were treated identically. The results (Fig. 4) show that the ROS-GC1 activity is stimulated by bicarbonate in a dose-dependent fashion. The stimulation is about 4-fold above the basal value (from 100 to ~ 400 pmol cyclic GMP $\text{min}^{-1} \text{mg}^{-1}$ prot) and it is comparable to that observed for ONE-GC (Fig. 2A). Also the EC_{50} for sodium bicarbonate of ~ 30 mM is within the range observed for stimulation of ONE-GC. As expected, membranes of control-transfected cells did not express guanylate cyclase activity and did not respond to bicarbonate (Fig. 4). These results show that the bicarbonate stimulatory effect on the ONE-GC activity is not unique; it also occurs with ROS-GC1.

It is noted that there are some significant contradictions in the two previous reports claiming that the bicarbonate modulation of ONE-GC is unique, [19–22]. One report [19] shows that, instead of stimulation, 50 mM bicarbonate inhibits basal activities of all members of the membrane guanylate cyclase: $\sim 20\%$ of ANF-RGC, $\sim 5\%$ of STa-RGC, $\sim 40\%$ of ROS-GC1 and $\sim 60\%$ of ROS-GC2. The other report [22] shows inhibition of the basal guanylate cyclase activity only for ANF-RGC; and for the other members of the membrane guanylate cyclase family, it shows no bicarbonate-dependent activity.

Discussion

This study contributes to our understanding of the emerging field of membrane guanylate cyclase linked with the odorant signaling. It demonstrates that bicarbonate, a mediator of the odorant atmospheric CO_2 signaling of ONE-GC is independent of Ca^{2+} . This makes ONE-GC a unique member of the membrane guanylate cyclase family because it is the only one that can, through distinct modes, transduce multiple, at least three, odorant signals into the production of their second messenger cyclic GMP. This shows how flexible this transduction system is and how radically different it is from the cyclic AMP transduction system linked with the odorant signaling. The first part of the discussion focuses on the new findings of the study and the latter parts on its present understanding.

To date ONE-GC is the only member of the membrane guanylate cyclase family that has been linked with the odorant signal transduction at the biochemical and the physiological level

[reviewed in: 4]. It represents the sole member of the crossover subfamily, belonging to both of its sister subfamilies, peptide hormone receptor and Ca^{2+} -modulated. Equipped with the former attribute, it is a direct receptor of the odorant peptide, uroguanylin [13, 14]; and consistent with the prototype model of ANF-RGC [17], upon binding uroguanylin at its extracellular domain it primes itself, yet gets only minimally activated [23]. This step is Ca^{2+} -independent [23]. Equipped with the latter attribute, at its intracellular domain through three Ca^{2+} sensors [23, 26, 27] it fully amplifies the odorant signal and at a defined site of the catalytic module translates the odorant signal in the production of its second messenger, cyclic GMP. Three recent studies have demonstrated its additional transduction feature, which is that it is also a transducer of the odorant atmospheric CO_2 [18, 19, 22]. ONE-GC senses CO_2 after its conversion by carbonic anhydrase to bicarbonate, which, in turn stimulates it directly at the intracellular domain. Based on the bicarbonate target site and the prior knowledge that ONE-GC transduction mechanism is Ca^{2+} -modulated, one would have envisioned that the bicarbonate-modulated transduction mechanism of this guanylate cyclase is also Ca^{2+} -dependent. The present finding demonstrates that this is not the case, however. It is Ca^{2+} -independent. Thus, contrary to the ROS-GC model, in this case ONE-GC does not require any of its Ca^{2+} sensors: neurocalcin δ [24], GCAP1 [26] or hippocalcin [27]. It is, therefore, concluded that the bicarbonate signaling of ONE-GC is novel, it is Ca^{2+} -independent, and through a novel mechanism ONE-GC becomes an independent transducer of an additional odorant, atmospheric CO_2 .

This conclusion created a puzzle. The prior studies had demonstrated that the bicarbonate signal transduction site resides in the catalytic domain of ONE-GC; one report, however, defined the catalytic domain as V⁵⁴⁶-G¹⁰⁷¹ [22] and the other, as e⁸⁵⁰-C¹¹¹⁰ segment of the guanylate cyclase [19]. The catalytic domain has been precisely defined and amino acid residues M⁸⁸⁰ and P¹⁰²⁸ determine its boundary [14]. Importantly, the Ca^{2+} -modulated neurocalcin δ signal transduction site covers the M⁸⁸⁰-L⁹²¹ segment of this domain. The puzzle was: how could this conserved among the membrane guanylate cyclases domain be the transducer of both Ca^{2+} -dependent and Ca^{2+} -independent signals?

This puzzle was solved by the studies that targeted the isolated catalytic domain, M⁸⁸⁰-P¹⁰²⁸. The neurocalcin δ -modulated Ca^{2+} signal transduction site in this domain is between M⁸⁸⁰-L⁹²¹. Analysis of the catalytic domain demonstrated that the bicarbonate site also resides in this domain of ONE-GC. Because the bicarbonate domain is Ca^{2+} -independent, the logic dictated that it would not overlap with the neurocalcin δ site.

This assumption was validated through the dissection of the catalytic domain. The motif ⁹⁰⁸LSEPIE⁹¹³ in the catalytic domain is critical for the Ca^{2+} -modulated signal transduction. However, absence of this motif does not affect the bicarbonate signaling of ONE-GC. These results demonstrate that the bicarbonate site is present in the catalytic domain of ONE-GC but it is different from the Ca^{2+} -signaling site. By exclusion, it is possible that the site follows the Ca^{2+} signal transduction motif and resides in the aa 922-1028 segment (Fig. 3D). These results also show that among membrane guanylate cyclases ONE-GC transduction machinery is uniquely flexible. It is a transducer of three odorants—green pepper [15, 16], uroguanylin [13, 14] and CO_2 [18]; and it adopts itself to processing these odorants in multiple modes—through its extracellular, intracellular, Ca^{2+} -dependent and Ca^{2+} -independent modes.

The identity of the core catalytic domain of ONE-GC as the bicarbonate signal transduction site provided an extraordinary insight into the bicarbonate general signaling mechanism of the membrane guanylate cyclases. This site is conserved in the membrane guanylate cyclase family, and is 85% conserved in the photoreceptor ROS-GC1. Logic dictated that ROS-GC1 should be responsive to the bicarbonate stimulation. Yet, the earlier reports claimed otherwise

[19–22], and in fact one report showed that the basal activity of ROS-GC1 was significantly inhibited by the bicarbonate [19].

These inconsistencies between the logic and the earlier results made the present authors revisit those results. The present findings show that ROS-GC1 is stimulated robustly by bicarbonate. Its EC₅₀ value is comparable to that of ONE-GC and like ONE-GC the stimulation of ROS-GC1 is Ca²⁺-independent. Thus, the bicarbonate signaling of ONE-GC is not unique. It exists also for ROS-GC1. Because the site is conserved, it is possible that other membrane guanylate cyclases may also respond to the bicarbonate signaling.

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Figure 1. Schematic representation of ONE-GC and its expression constructs

The following abbreviations denote the predicted domains: ls, leader sequence; ext, extracellular domain; tm, transmembrane domain; jmd, juxtamembrane domain; khd, kinase homology domain; dd, putative dimerization domain; catd, cyclase catalytic domain; cte, C-terminal extension. Specific basal guanylate cyclase activity of ONE-GC and of the mutants expressed in COS cells is given in the right-hand column [A].

Figure 2. Ca^{2+} independent stimulation of ONE-GC by bicarbonate (A) and Ca^{2+} -dependent stimulation by neurocalcin δ (B)

Membranes of COS cells expressing ONE-GC were exposed to indicated concentrations of sodium bicarbonate (A) or neurocalcin δ (B) in the presence of 10 μM Ca^{2+} or 1 mM EGTA. In control experiment membranes of COS cells transfected with empty pcDNA3 vector were treated with bicarbonate. **The ONE-GC core catalytic domain is modulated by both sodium bicarbonate and neurocalcin δ (C).** ONE-GC deletion mutant tm-catd was expressed in COS cells and their membranes were exposed to increasing concentrations of sodium bicarbonate or neurocalcin δ and 10 μM Ca^{2+} . All experiments were done in triplicate and repeated three times with separate membrane preparations. The results presented (mean $\pm$ SD) are from one representative experiment.

Figure 3. Bicarbonate and neurocalcin δ target different sites of ONE-GC core catalytic domain (A) Role of the $^{908}\text{LSEPIE}^{913}$ sequence motif in neurocalcin δ and (B) bicarbonate dependent ONE-GC activity. The ONE-GC Δ LSEPIE mutant was expressed in COS cells and the guanylate cyclase activity was determined in the presence of indicated concentrations of neurocalcin δ and 10 μM Ca^{2+} or sodium bicarbonate. Membranes of COS cells expressing full-length ONE-GC were treated identically as positive control. (C) Additive effects of neurocalcin δ and bicarbonate on ONE-GC activity. Membranes of COS cells expressing ONE-GC were treated with 2 μM neurocalcin δ , 30 mM sodium bicarbonate or simultaneously by both in the presence of 10 μM Ca^{2+} and assayed for guanylate cyclase activity. The experiments were done in triplicate and repeated two times with separate membranes preparations and the results presented (mean $\pm$ SD) are from these experiments. (D) Schematic representation of ONE-GC regions targeted by neurocalcin δ and bicarbonate. The neurocalcin δ (NC) and bicarbonate (HCO_3^-) signaling sites are located in tandem within its core catalytic domain. The neurocalcin δ site is located within the M 880 -L 921 region and the bicarbonate site is hypothesized to be located within the Y 922 -P 1028 region. The abbreviations name the topographic domains of ONE-GC: EXT, extracellular; TM, transmembrane domain; JMD, juxtamembrane domain; KHD, kinase homology domain; DD, hypothetical dimerization domain; CCD, core catalytic domain; CTE, C-terminal extension. For convenience, the EXT dimension is not proportionate.

Figure 4. Bicarbonate effect on the activity of ROS-GC1

Membranes of COS cells expressing ROS-GC1 were exposed to the indicated concentrations of sodium bicarbonate and assayed for guanylate cyclase activity. COS cells transfected with empty pcDNA3 vector were used as control. The experiment was done in triplicate and repeated three times with separate membrane preparations. The results presented (mean $\pm$ SD) are from these experiments.