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Sulfonylurea effects on acid and pepsinogen secretion in isolated rabbit gastric glands

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Abstract

The influence of different sulfonylureas on the rate of acid and pepsinogen secretion was studied in isolated rabbit gastric glands. Neither tolbutamide (10–500 μM), chlorpropamide (10–500 μM), glibenclamide (1–50 μM) nor glipizide (1–50 μM) exerted a secretory effect. In contrast, gliquidone caused a marked and dose-dependent stimulation of acid production in gastric glands incubated under basal conditions and potentiated the stimulatory effect of both histamine and carbachol. Gliquidone also increased the rate of pepsinogen release in gastric glands incubated either under basal conditions or in the presence of cholecystokinin-octapeptide or isoproterenol. The secretory effects of gliquidone were associated with a significant increase in the glandular content of cyclic AMP, caused by a competitive inhibition of low-K_m cyclic AMP phosphodiesterase. Our results indicate that, among the assayed sulfonylureas, only gliquidone, in the micromolar range, stimulates acid and pepsinogen secretion through a cyclic AMP-dependent mechanism. © 1998 Elsevier Science B.V.

Keywords: Acid, gastric; Gastric gland; Gliquidone; Pepsinogen; Sulfonylurea

1. Introduction

Sulfonylureas are oral hypoglycemic agents widely used in the treatment of type II diabetes mellitus (Melander et al., 1990). Both pancreatic and extrapancreatic actions have been reported for these agents (Feldman and Lebovitz, 1969; Beck-Nielsen et al., 1988; Henquin, 1992). The most relevant pancreatic action is the direct and immediate stimulation of insulin release. In the β-cell, sulfonylureas bind to high-affinity specific receptors that are closely related to ATP-dependent K^+ channels, resulting in a blockade of K^+ efflux. This leads to the depolarization of the plasma membrane and to the subsequent influx of extracellular Ca^{2+} through voltage-dependent Ca^{2+} channels. An increase in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) is the signal that triggers insulin release (Boyd et al., 1990; Malaisse and Lebrun, 1990). ATP-dependent K^+ channels, whose activity can be modulated by sulfonylureas, have also been identified in different excitable tissues such as heart, brain or smooth muscle (Ashcroft and Ashcroft, 1992; Panten et al., 1992). Moreover, sulfonylureas have also been reported to increase $[Ca^{2+}]_i$ in non-excitable cells like hepatocytes or adipocytes. In these cells, the increase in $[Ca^{2+}]_i$ is related to the acceleration of extracellular Ca^{2+} influx, seemingly without participation of ATP-sensitive K^+ channels (Draznim et al., 1987; López-Alarcón et al., 1993).

Several reasons led us to carry out a systematic study of the influence of different sulfonylureas on acid and pepsinogen secretion in isolated rabbit gastric glands. First, as mentioned above, sulfonylureas may modulate $[Ca^{2+}]_i$ in different types of excitable and non-excitable cells. Second, it is well established that secretagogues like carbachol, which raises $[Ca^{2+}]_i$ in parietal cells, or cholecystokinin, which modulates $[Ca^{2+}]_i$ in chief cells, stimulate

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acid and pepsinogen secretion, respectively, in isolated rabbit gastric glands (Hersey, 1987; Soll and Berglindh, 1987). Finally, gastrointestinal complaints, such as nausea and vomiting, have been reported among the untoward responses to sulfonylureas (Lebovitz and Feinglos, 1978; Martindale, 1996).

We found that among the assayed sulfonylureas (tolbutamide, chlorpropamide, glipizide, gliquidone and glibenclamide), only gliquidone, in the micromolar range, caused a significant stimulation of the rate of both acid and pepsinogen secretion in isolated rabbit gastric glands. The secretory effect of gliquidone appeared to be related to an increase in the glandular concentration of cyclic AMP, as a result of competitive inhibition of low-K_m cyclic AMP phosphodiesterase activity.

2. Materials and methods

2.1. Animals

Male rabbits (New Zealand, 1.5–2.5 kg body weight) were obtained from our inbred colony. The animals were fed on standard chow (N-25 Moragón, Toledo, Spain) and water ad libitum.

2.2. Reagents

Collagenase A from *Clostridium histolyticum* (type I), histamine dihydrochloride, carbamylcholine chloride, isoproterenol hydrochloride, forskolin, 3-isobutyl-1-methylxanthine (IBMX) and snake (*Ophiophagus hannah*) venom were obtained from Sigma Chemical Co. (St. Louis, MO). Nonsulfated cholecystokinin octapeptide (CCK-OCT) was purchased from Bachem Feinchemikalien (Bubendorf, Switzerland). Gliquidone (lot 228878), glipizide (lot MP-605/7143) and chlorpropamide (lot 0014) were kindly supplied by Boehringer Ingelheim España (Barcelona, Spain), Farmitalia-Carlo Erba (Barcelona, Spain) and Pfizer (Madrid, Spain), respectively. Glibenclamide (lot 15931) and tolbutamide (lot M-86-0044) were gifts from Boehringer Mannheim (Germany). Anion-exchange resin (AG 1-X8, 200–400 mesh, chloride form) was from Bio-Rad Laboratories (Madrid, Spain). [Dimethyl-amine-^{14}C]aminopyrine (100–120 mCi/mmol), [2,8-^{3}H]cAMP (30–50 Ci/mmol), $^{45}CaCl_2$ and the cyclic AMP ^{125}I-radioimmunoassay kit were purchased from Amersham International (Buckinghamshire, England). The remaining reagents, all of analytical grade, were from Boehringer, Sigma or Merck (Darmstadt, Germany).

2.3. Gastric gland isolation

Gastric glands were obtained according to the method reported by Berglindh and Öbrink (1976), as previously described (Rossi et al., 1992). Briefly, after anesthesia with sodium pentobarbital (30 mg/kg body weight, intravenously), the stomach was perfused in situ with phosphate-buffered saline (149.6 mM NaCl, 3 mM K_2HPO_4 and 0.64 mM NaH_2PO_4, pH 7.4) at 37°C. The mucosa from the gastric corpus of the stomach was then freed from the muscular layers, minced and incubated with collagenase (1 mg/ml) at 37°C for 30–45 min. The isolated gastric glands were filtered through nylon mesh, rinsed three times and suspended, at a final concentration of 2 to 5 mg dry weight/ml, in medium A (132.4 mM NaCl, 5.4 mM KCl, 5 mM Na_2HPO_4, 1 mM NaH_2PO_4, 1.2 mM $MgSO_4$, 1 mM $CaCl_2$, 0.5 mM dithiothreitol, 15 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethane-sulfonic acid (HEPES), 10 mg/l phenol red, 10 mM glucose and 1 mg/ml bovine serum albumin, at pH 7.3). The incubations were performed with agitation (100 strokes/min), at 37°C, in an O_2/CO_2 atmosphere (95%/5%). Sulfonylureas were dissolved in 5 mM NaOH. Control incubations of gastric glands were carried out in the presence of NaOH at similar final concentrations. No significant differences were observed between control and saline (0.9% NaCl) incubations with respect to any assayed metabolic parameter.

Isolated rabbit gastric glands had a typical rod-like appearance (0.3–0.7 mm in length). More than 95% of the different cells present in the glands excluded the Trypan blue dye (1%). The dry weight of the gastric gland suspensions was calculated for 1 ml aliquots; after centrifugation ($10\,000 \times g$ for 3 min), the pellets were dried overnight at 60°C and then weighed.

2.4. Measurement of acid production and pepsinogen release

Acid production was determined by the accumulation of [^{14}C]aminopyrine in the canalicular compartment, according to the method described by Berglindh et al. (1976), with minor modifications. Briefly, 1.5 ml samples of gland suspensions were incubated in medium A, as indicated above, in the presence of 0.88 μM [^{14}C]aminopyrine (0.1 μCi/ml) and different agents. After a 30 min incubation, 1 ml aliquots of gland suspensions were taken and immediately centrifuged ($10\,000 \times g$ for 20 s). The supernatants were quickly removed and the pellets were rinsed twice with medium A, dried and dissolved in 250 μl of 60% HNO_3, at 50°C for 15 min. The radioactivity of the supernatants and of acid extracts was counted in a liquid scintillation spectrometer. Results are expressed as the [^{14}C]aminopyrine concentration ratio between intraglandular and extraglandular water, which was calculated as described elsewhere (Berglindh et al., 1976; Rossi et al., 1992).

The secretion of pepsinogen was estimated as previously described (Rossi et al., 1992). Briefly, gland suspensions were incubated, at 37°C, in a shaking water bath for 30 min in the presence of different agents. At zero time and at the end of the incubation, 1 ml samples of gland

suspensions were taken and centrifuged (10 000 × *g* for 15 s). Pepsinogen was measured in aliquots of the supernatants by the method reported by Hersey et al. (1983). Total pepsinogen was determined in samples of the incubations treated with 0.25% Triton X-100. Pepsinogen released into the medium is expressed as a percentage of total pepsinogen present in the incubations.

2.5. $^{45}Ca^{2+}$ uptake by the gastric glands

Ca^{2+} uptake studies were carried out following the method described by Kimmich (1975), with minor modifications. Gland suspensions were preincubated in medium A at 37°C for 5 min. At zero time, $^{45}CaCl_2$ (1 μCi/ml) was added together with tolbutamide, chlorpropamide, glibenclamide, glipizide, gliquidone or saline. At selected times (15, 30, 60 and 120 s of incubation), samples of gland incubations were pipetted into ice-cooled tubes containing 8 ml of solution B (10 mM HEPES, 150 mM NaCl, 5 mM $CaCl_2$ and 0.1 mM $LaCl_3$, at pH 7.4) and were immediately filtered under vacuum (25 cmHg) through cellulose filters (Millipore 0.65 μm pore). Gastric glands retained by the filters were washed with 15 ml of medium B, in order to eliminate the unincorporated radioactivity. The filters were dried at 60°C for 30 min and their radioactivity was measured in a liquid scintillation spectrometer.

2.6. Assays of cyclic AMP phosphodiesterase activity and cyclic AMP

Low-K_m cyclic AMP phosphodiesterase activity of gastric glands was determined according to the two-step isotopic procedure described by Thompson et al. (1979). The method consists of the hydrolysis of [^{3}H]cyclic AMP to [^{3}H]5′-AMP by phosphodiesterase activity. The resulting [^{3}H]5′-AMP is then further hydrolyzed to [^{3}H]adenosine by addition of 5′-nucleotidase from snake venom. Samples are then filtered through an anion-exchange resin (Dowex 1-X8, 200–400 mesh) to bind all charged nucleotides, leaving [^{3}H]adenosine as the only labelled compound to be counted. To assay gastric gland cyclic AMP phosphodiesterase, isolated gastric glands (about 0.6 g wet weight) were gently homogenized in three volumes of a medium containing 10 mM Tris–HCl pH 7.4 and 0.25 M sucrose, with the use of a motor-driven glass–Teflon homogenizer. The homogenate was centrifuged at 12 000 × *g* for 20 min, at 4°C. The resulting supernatant was subsequently centrifuged at 105 000 × *g* for 60 min. Aliquots of the latter supernatant were used to assay phosphodiesterase activity. The reaction mixture contained 40 mM Tris–HCl pH 8.0, 10 mM $MgCl_2$, 3.75 mM β-mercaptoethanol, 125 μg/ml bovine serum albumin, 0.35 μM cyclic AMP (0.32 μCi/nmol) and 0.03 mg of cytosolic protein from gastric gland extracts. The reaction was carried out at 30°C for 10 min and then the samples were processed as indicated by Thompson et al. (1979).

The glandular content of cyclic AMP was determined in trichloroacetic acid extracts of gastric gland suspensions by using a radioimmunological method, as previously described (Felíu et al., 1983). Gastric glands were incubated, at 37°C, for 5 min with different concentrations of sulfony-

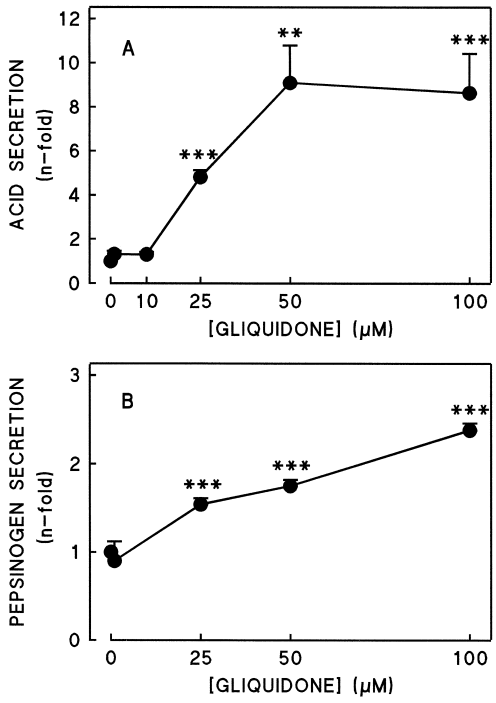

Fig. 1. Effect of different concentrations of gliquidone on the rates of acid (A) and pepsinogen (B) secretion in isolated rabbit gastric glands. Gastric glands were incubated for 30 min with different concentrations of gliquidone, as indicated in Section 2. Control values were 1.41 ± 0.09 for acid secretion and 6.54 ± 1.10 for pepsinogen secretion. Values represent the mean ± S.E.M. of seven different experiments, with incubations carried out in duplicate. Paired Student's *t*-test versus the corresponding control value: $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 1
Effect of sulfonylureas on acid and pepsinogen secretion in isolated rabbit gastric glands

	Acid secretion	Pepsinogen secretion
Control	1.00 ± 0.0	1.00 ± 0.0
Glibenclamide, 1 μM	1.03 ± 0.15^{NS}	1.04 ± 0.06^{NS}
Glibenclamide, 50 μM	0.88 ± 0.10^{NS}	1.07 ± 0.06^{NS}
Tolbutamide, 10 μM	1.05 ± 0.16^{NS}	1.14 ± 0.07^{NS}
Tolbutamide, 500 μM	0.93 ± 0.16^{NS}	1.01 ± 0.14^{NS}
Chlorpropamide, 10 μM	0.76 ± 0.23^{NS}	1.27 ± 0.19^{NS}
Chlorpropamide, 500 μM	0.82 ± 0.05^{NS}	0.83 ± 0.19^{NS}
Glipizide, 1 μM	0.69 ± 0.38^{NS}	0.95 ± 0.17^{NS}
Glipizide, 50 μM	1.13 ± 0.16^{NS}	0.97 ± 0.09^{NS}

Isolated gastric glands were incubated for 30 min with different concentrations of sulfonylureas, as indicated in Section 2. Results are expressed as stimulation (*n*-fold) versus the corresponding control value. Control acid secretion was 1.41 ± 0.09 μM and 6.54 ± 1.10 μM. Data are the means ± S.E.M. of four different experiments.
^{NS}: No significant difference versus the corresponding control incubation.

lureas, in the absence or in the presence of either IBMX (0.5 mM) or forskolin (10 μM). After deproteination with 5% trichloroacetic acid and subsequent centrifugation ($3000 \times g$ for 15 min), cyclic AMP was determined in the supernatants.

2.7. Statistical analysis

Statistical significance of differences was calculated by the paired Student's *t*-test. The differences were considered statistically significant when P was less than 0.05. The K_i value was calculated by the FIG.P figure processor.

3. Results

In a systematic study of the influence of different sulfonylureas on the rate of acid formation and pepsinogen secretion in isolated rabbit gastric glands, we observed that the presence of glibenclamide, tolbutamide, chlorpropamide or glipizide in the incubation medium did not significantly modify the basal rate of either acid production or pepsinogen release (Table 1). Among the sulfonylureas assayed, only gliquidone clearly stimulated acid production in a dose-dependent manner, in isolated gastric glands (Fig. 1A). The maximal stimulation (9.10 ± 1.69-fold versus basal incubation) was already observed at 50 μM, the calculated concentration corresponding to the half-maximal effect (EC_{50}) being 30 μM. Gliquidone also stimulated the release of pepsinogen in isolated gastric glands. In fact, a 2.38 ± 0.08-fold greater release of pepsinogen was observed with 100 μM gliquidone than under basal conditions (Fig. 1B).

The influence of gliquidone on acid production in either histamine- or carbachol-stimulated gastric glands was also studied. As expected, histamine increased the rate of acid production in a dose-dependent manner (Fig. 2A); the maximal stimulatory effect (8.57 ± 1.50-fold versus the basal value) was observed at 100 μM histamine. The additional presence of gliquidone in the incubation medium

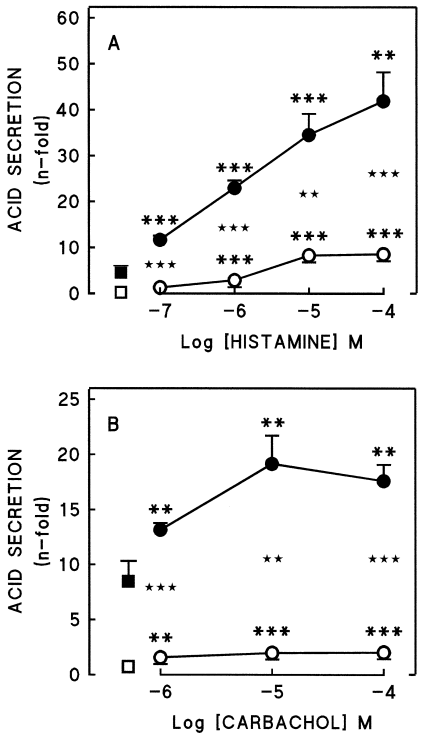

Fig. 2. Influence of gliquidone on the rate of acid production in isolated rabbit gastric glands stimulated by either histamine or carbachol. Gastric glands were incubated for 30 min, at 37°C, either under basal conditions (□, ■) or with different concentrations of histamine (A) or carbachol (B), in the absence (open symbols) or in the presence of 50 μM gliquidone (full symbols). The [^{14}C]aminopyrine concentration ratio under basal conditions was 1.21 ± 0.1 for (A) and 1.52 ± 0.13 for (B). Values represent the mean $\pm$ S.E.M. of four different experiments, with incubations carried out in duplicate. Paired Student's *t*-test: $^{**}P < 0.01$ and $^{***}P < 0.001$, versus the corresponding control value; $^{\star\star}P < 0.01$ and $^{\star\star\star}P < 0.001$, versus the corresponding histamine or carbachol incubations.

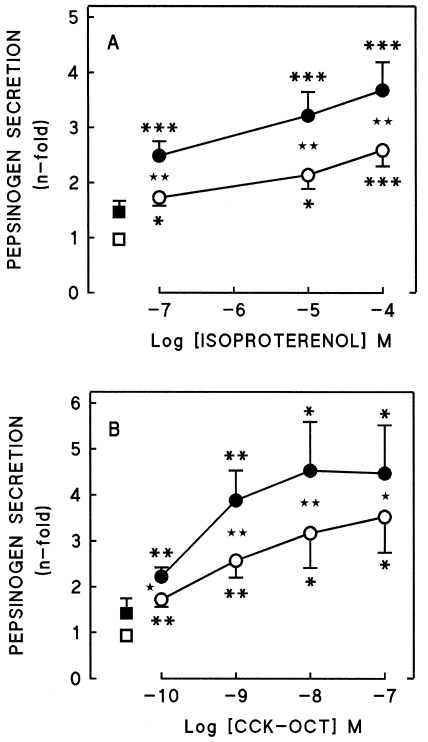

Fig. 3. Influence of gliquidone on the rate of pepsinogen release from isolated rabbit gastric glands stimulated by either isoproterenol or CCK-OCT. Gastric glands were incubated for 30 min at 37°C, either under basal conditions (□, ■) or with different concentrations of isoproterenol (A) or CCK-OCT (B), in the absence (open symbols) or in the presence of 50 μM gliquidone (full symbols). Pepsinogen release under basal conditions was 10.8 ± 1.75 for (A) and 6.76 ± 1.28 for (B). Values represent the mean $\pm$ S.E.M. of four to seven different experiments, with incubations carried out in duplicate. Paired Student's *t*-test: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ versus the corresponding control value; $^{\star}P < 0.05$ and $^{\star\star}P < 0.01$, versus the corresponding isoproterenol or CCK-OCT incubations.

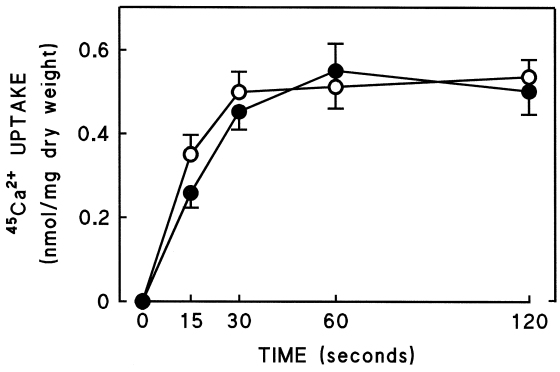

Fig. 4. Time course of the effect of gliquidone on $^{45}Ca^{2+}$ uptake in isolated rabbit gastric glands. Gastric glands were incubated, at 37°C, in the absence (○) or in the presence of 50 μM gliquidone (●) for the selected times. Samples of the gastric gland incubations were processed as indicated in Section 2. Values are the means ± S.E.M. of four different experiments, with incubations being carried out in duplicate.

clearly potentiated the stimulation of acid secretion caused by this agent. In fact, the simultaneous presence of gliquidone (50 μM) and histamine (100 μM) in the incubation medium resulted in a 41.91 ± 6.30-fold increase in the rate of acid production, as compared to that observed under basal conditions (Fig. 2A). Carbachol also significantly raised the rate of acid production (2.03 ± 0.16-fold at 100 μM). The additional presence of gliquidone clearly potentiated the effect of this secretagogue; thus, the simultaneous presence in the incubation medium of both gliquidone and carbachol (50 and 100 μM, respectively) raised the basal rate of acid production 17.61 ± 1.47-fold (Fig. 2B).

We also studied the influence of gliquidone on the rate of pepsinogen release stimulated by either isoproterenol or CCK-OCT. As shown in Fig. 3, these two agents caused a dose-dependent stimulation of the rate of pepsinogen release. The presence of 100 μM isoproterenol or 10 nM CCK-OCT in the incubation medium caused a significant increase in the rate of pepsinogen release by isolated gastric glands (2.59 ± 0.29- and 3.17 ± 0.86-fold greater versus the basal value, respectively). Again, the additional presence of gliquidone (50 μM) significantly reinforced the actions of both isoproterenol and that of CCK-OCT, resulting in a 3.68 ± 0.51- and 4.53 ± 1.06-fold greater stimulation, respectively, as compared to the corresponding basal values.

To gain more insight into the mechanism by which gliquidone stimulates acid secretion and pepsinogen release, we studied the influence of this sulfonylurea on the rate of $^{45}Ca^{2+}$ uptake by isolated gastric glands, as well as on the glandular levels of cyclic AMP. As shown in Fig. 4, gliquidone (50 μM) did not significantly affect the basal rate of $^{45}Ca^{2+}$ uptake during the entire incubation time. Moreover, no significant changes in Ca^{2+} uptake were obtained in the presence of other sulfonylureas (glibenclamide, tolbutamide, chlorpropamide and glipizide) (data not shown). Neither glibenclamide, tolbutamide, chlorpropamide nor glipizide significantly modified the glandular concentration of cyclic AMP (control: 2.88 ± 0.26; 50 μM glibenclamide: 2.84 ± 0.34^{NS}; 100 μM tolbutamide: 2.70 ± 0.31^{NS}; 100 μM chlorpropamide: 2.88 ± 0.09^{NS}; 50 μM glipizide: 2.64 ± 0.32^{NS}. These values represent the mean ± S.E.M. of 4 different experiments and correspond to pmol of cyclic AMP/mg dry weight of gastric glands). In contrast, gliquidone (50 μM) significantly raised the glandular content of cyclic AMP by about 25% over the control value (Table 2). The stimulatory effect of gliquidone on cyclic AMP levels was also observed in gastric glands incubated with histamine, isoproterenol, carbachol or CCK-OCT (Table 2). As previously reported (Hersey, 1987; Soll and Berglindh, 1987), these last two secretagogues did not significantly affect the glandular concentration of cyclic AMP, when assayed separately.

The rise in the glandular content of cyclic AMP caused by gliquidone could be explained by either stimulation of cyclic AMP production or inhibition of cyclic AMP degradation. To determine which of these two possible mechanisms was implicated, the influence of gliquidone (0-100 μM) on cyclic AMP levels was studied in isolated gastric glands incubated either in the presence of 10 μM forskolin (a known activator of adenylate cyclase) or in the presence of 0.5 mM IBMX (an inhibitor of cyclic AMP phosphodiesterase activity). As shown in Fig. 5, under basal conditions the increase in cyclic AMP levels was dependent on the assayed concentration of sulfonylurea; the maximal effect (35% over basal value) was observed at the highest gliquidone concentration (100 μM) (basal: 2.17 ± 0.19; 100 μM gliquidone: 2.93 ± 0.15 ($P < 0.01$)). It is noteworthy that the effect of the sulfonylurea persisted in the

Table 2
Effect of gliquidone on cyclic AMP levels in isolated rabbit gastric glands incubated with different secretagogues

	Saline	50 μM gliquidone
Control	2.88 ± 0.26	3.57 ± 0.30^{A}
Histamine 100 μM	4.06 ± 0.39^{b}	5.80 ± 0.46^{Ba}
Carbachol 100 μM	2.91 ± 0.27^{NS}	4.13 ± 0.24^{A}
CCK-OCT 0.1 μM	3.06 ± 0.51^{NS}	4.45 ± 0.47^{A}
Isoproterenol 10 μM	5.35 ± 0.89^{a}	11.37 ± 1.98^{Aa}

Isolated gastric glands were incubated with the indicated agents for 5 min at 37°C. Cyclic AMP was determined in acid extracts by a radioimmunological method. Results are expressed as pmol of cyclic AMP per mg of dry weight. Values are the means ± S.E.M. of four different experiments.
[A] Paired Student's *t*-test versus the corresponding saline incubation: $P < 0.05$.
[B] Paired Student's *t*-test versus the corresponding saline incubation: $P < 0.01$.
[NS]: No significant difference.
[a] Paired Student's *t*-test versus the corresponding control incubation: $P < 0.05$.
[b] Paired Student's *t*-test versus the corresponding control incubation: $P < 0.01$.

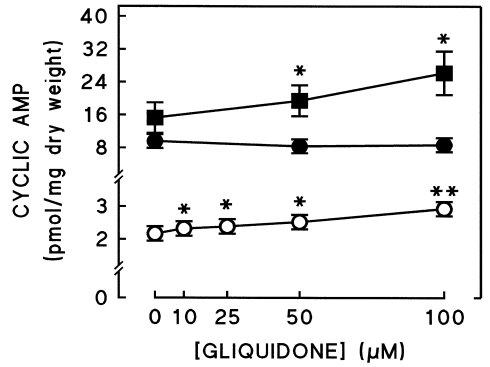

Fig. 5. Effect of different concentrations of gliquidone on cyclic AMP levels in isolated rabbit gastric glands. Gastric glands were incubated, at 37°C for 5 min, in the absence (○) or in the presence of either 0.5 mM IBMX (●) or 10 μM forskolin (■). Values represent the mean ± S.E.M. of four different experiments, with incubations being carried out in duplicate. Paired Student's *t*-test versus the corresponding control value: $^{*}P < 0.05$; $^{**}P < 0.01$.

presence of forskolin (10 μM forskolin: 15.28 ± 4.6; 10 μM forskolin + 100 μM gliquidone: 26.21 ± 7.48 ($P < 0.05$)), while it was suppressed in gastric glands treated with IBMX (0.5 mM IBMX: 9.59 ± 2.06; 0.5 mM IBMX + 100 μM gliquidone: 8.71 ± 1.59 (no significant difference)). These results suggest that gliquidone may raise the glandular levels of this cyclic nucleotide by inhibition of cyclic AMP phosphodiesterase activity. In fact, gliquidone caused a dose-dependent inhibition of the activity of this enzyme in cytosolic extracts of isolated rabbit gastric glands. The maximal inhibitory effect (about 85%) was observed at 100 μM gliquidone (basal: 126.28 ± 5.66; 100 μM gliquidone: 15.17 ± 0.63 ($P < 0.001$)), the calculated EC_{50} value corresponding to 7 μM (Fig. 6). The inhibition of cyclic AMP phosphodiesterase activity caused by 0.5 mM IBMX (about 92%) is also shown in Fig. 6.

In order to elucidate the type of inhibition of cyclic AMP phosphodiesterase activity exerted by gliquidone, we

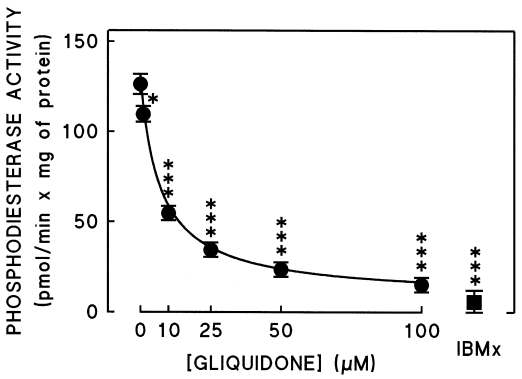

Fig. 6. Effect of different concentrations of gliquidone on cyclic AMP phosphodiesterase activity in isolated rabbit gastric glands. Enzyme activity was assayed in gastric gland extracts, as indicated in Section 2. Values represent the means ± S.E.M. of four different experiments. The inhibitory effect of IBMX (0.5 mM) was also tested (■). Paired Student's *t*-test versus the basal value: $^{*}P < 0.05$; $^{***}P < 0.001$.

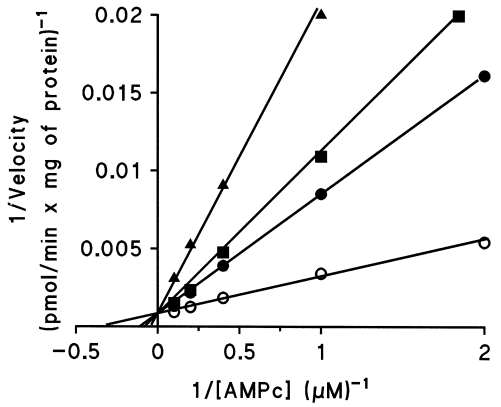

Fig. 7. Double-reciprocal plot of the inhibition of cyclic AMP phosphodiesterase by gliquidone. Concentrations of gliquidone: none (○), 10 μM (●), 25 μM (■) and 100 μM (▲). Data are from a representative experiment carried out in triplicate.

carried out a kinetic analysis in the presence of different concentrations of this sulfonylurea. As shown in Fig. 7, gliquidone caused a competitive inhibition of low-K_m cyclic AMP phosphodiesterase activity, the calculated K_i being 7.1 ± 1.3 μM. In contrast, neither chlorpropamide, glipizide, nor glibenclamide significantly modified the activity of this enzyme. Only tolbutamide caused a small but statistically significant stimulation of cyclic AMP phosphodiesterase activity (control: 126.28 ± 5.66; 100 μM chlorpropamide: 161.71 ± 17.80^{NS}; 50 μM glibenclamide: 110.0 ± 1.40^{NS}; 100 μM tolbutamide: 173.80 ± 17.91 ($P < 0.05$); values are the means ± S.E.M. from 6 experiments and are expressed in pmol/mg of protein × min).

4. Discussion

In addition to the stimulation of insulin release, sulfonylureas also exert metabolic effects, related and not related to its hypoglycemic action, in extrapancreatic tissues (Feldman and Lebovitz, 1969; Beck-Nielsen et al., 1988; Melander et al., 1990). Thus, increase in cardiac contractility (Tan et al., 1984), inhibition of platelet aggregation (Levey, 1977), stimulation of gastric secretion (Roth et al., 1971) and alteration of water balance (Skillman and Feldman, 1981) are some of the extrapancreatic effects of sulfonylureas not related to the control of glycemia.

The mechanism by which sulfonylureas exert their effects in extrapancreatic tissues is controversial. In excitable tissues such as heart, brain or smooth muscle (Ashcroft and Ashcroft, 1992; Panten et al., 1992), sulfonylureas have been reported to block ATP-dependent K^+ channels, causing the depolarization of the cell membrane and the subsequent influx of extracellular Ca^{2+} through voltage-operated Ca^{2+} channels. In contrast, in isolated rat hepatocytes and adipocytes, sulfonylureas have been shown to increase $[Ca^{2+}]_i$ and to accelerate the uptake of extracellular $^{45}Ca^{2+}$ by a still to be definitively established mecha-

nism, which appears to be independent of the modulation of ATP-dependent K^+ channels (Draznim et al., 1987; López-Alarcón et al., 1993). Contradictory results have been published concerning the modulation by sulfonylureas of cyclic AMP levels, adenylate cyclase and cyclic AMP phosphodiesterase activities in different mammalian tissues. Thus, tolbutamide has been shown to activate adenylate cyclase and increase cyclic AMP levels in pancreas (Kuo et al., 1973). In addition, tolbutamide and other first-generation sulfonylureas are reported to cause a marked activation of adenylate cyclase in particulate preparations from both rabbit and human heart (Levey et al., 1971; Lasseter et al., 1972). Other studies have shown that sulfonylureas inhibit cyclic AMP-specific phosphodiesterase activity in a wide variety of tissues including adipose tissue, pancreas, brain, heart, kidney, liver and platelets (Brooker and Fichman, 1971; Goldfine et al., 1971; Roth et al., 1971). In contrast, several reports indicate that these drugs can reduce the tissue content of cyclic AMP through the activation of low-K_m cyclic AMP phosphodiesterase (Osegawa et al., 1982; Müller et al., 1994). Differences in the sulfonylureas, concentrations and mammalian tissues used may explain such discordant findings.

In this work, we studied the influence of different oral hypoglycemic agents (tolbutamide, chlorpropamide, glibenclamide, gliquidone and glipizide) on the rate of acid production and pepsinogen release in isolated rabbit gastric glands. Among the sulfonylureas tested, only gliquidone in the micromolar range was able to stimulate significantly the basal rate of acid production. In fact, in gastric glands incubated under basal conditions, the maximal stimulatory effect elicited by gliquidone was already observed at a concentration of sulfonylurea of 50 μM. This maximal effect was quantitatively similar to the stimulation caused by a saturating concentration of histamine and much higher than the maximal stimulation elicited by carbachol. Gliquidone also markedly potentiated the rate of acid production in gastric glands incubated in the presence of either histamine or carbachol, even at saturating concentrations of these two secretagogues. This does not support a possible indirect secretory action of gliquidone on acid formation through the release of histamine by mast cells present in the gastric gland preparations. Furthermore, the evidence for a direct effect of the sulfonylurea on gastric glands is reinforced by the observation that gliquidone also significantly stimulated the rate of pepsinogen release under basal conditions and in the presence of isoproterenol or CCK-OCT.

In an attempt to investigate the mechanism by which gliquidone stimulates the rates of acid and pepsinogen secretion, we studied the influence of these agents on both the rate of $^{45}Ca^{2+}$ uptake and on cyclic AMP levels in isolated gastric glands. Thus, it was observed that neither gliquidone or any of the other assayed sulfonylureas significantly modified the rate of $^{45}Ca^{2+}$ uptake by the glands. This indicates that the specific secretory effect of gliquidone appears not to be related to the modulation of Ca^{2+} influx into the gastric glands. When the influence of sulfonylureas on the glandular content of cyclic AMP was studied, it was observed that only gliquidone was able to increase the basal content of cyclic AMP in isolated gastric glands, an effect that was dose dependent. In gastric glands incubated under basal conditions, the increase caused by gliquidone in cyclic AMP levels was small, but statistically significant, and quite similar to that elicited by a saturating concentration of histamine, a known gastric acid secretagogue which operates through a cyclic AMP-dependent mechanism (Soll and Berglindh, 1987). Furthermore, the effect of gliquidone on the glandular levels of cyclic AMP was more clearly observed when the formation of the cyclic nucleotide was stimulated by the presence of either isoproterenol or histamine in the incubation medium. These findings, together with the fact that the effect of gliquidone persisted in the presence of 10 μM forskolin and disappeared in gastric glands treated with 0.5 mM IBMX, suggested that gliquidone could interfere with the degradation of cyclic AMP. Confirming this hypothesis, gliquidone, but none of the other sulfonylureas assayed, caused a marked and competitive inhibition of low-K_m cyclic AMP phosphodiesterase activity present in the soluble fraction of isolated rabbit gastric glands. The maximal inhibition (about 85%) was observed at 100 μM, the calculated K_i value being 7.1 ± 1.3 μM. Although, as previously mentioned, the subject is controversial, these findings agree with other reports showing the ability of sulfonylureas to cause competitive inhibition of cyclic AMP phosphodiesterase activity in different animal tissue extracts (Brooker and Fichman, 1971; Goldfine et al., 1971; Roth et al., 1971).

The pharmacological implications of our findings deserve a final comment. As shown above, gliquidone at concentrations ranging from 10 to 100 μM was able to significantly stimulate both acid and pepsinogen secretion and to potentiate the effect of well established secretagogues in an in vitro preparation of rabbit gastric glands. In humans, the maximal plasma concentration of gliquidone after a single oral dose (30 mg) is about 1 μM (Ferner and Chaplin, 1987), a concentration that is apparently without effect on acid and pepsinogen secretion. Nevertheless, it should be taken into account that much higher concentrations (theoretically near 0.3 mM) could be attained in the gastric lumen after oral administration of the drug. Our work stresses the importance of specific collateral biochemical effects, even within a given family of drugs.

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