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Ciclazindol Inhibits ATP-Sensitive K^+ Channels and Stimulates Insulin Secretion in CRI-G1 Insulin-Secreting Cells

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SUMMARY

Ciclazindol, an anorectic drug, was shown to inhibit ATP-sensitive K^+ (K_{ATP}) channel currents and stimulate insulin secretion from CRI-G1 insulin-secreting cells. In contrast, the structurally related anorectic agent mazindol and the amphetamine-based anorectic compounds diethylpropion, fenfluramine, and phentermine had no effect on K_{ATP} channel activity in this cell line. Similarly, ciclazindol elicited insulin secretion from CRI-G1 cells, whereas mazindol had no secretagogue action. The mechanism by which ciclazindol acts to inhibit K_{ATP} channel activity is different than that of the sulfonylureas as ciclazindol is effective after procedures that decouple the sulfonylurea receptor from the K_{ATP} channel. In agreement with this finding, ciclazindol failed to displace [^{3}H]glibenclamide from CRI-G1 microsomal membranes. Further experiments demonstrated that ciclazindol has no significant effect on voltage-activated currents in this cell line.

K_{ATP} channels play a central role in the control of insulin secretion from the pancreatic β cell (1). Closure of these channels leads to membrane depolarization, activation of voltage-dependent calcium channels, and, ultimately, the exocytotic release of insulin from secretory granules (2). Drugs that alter K_{ATP} channel function are therefore potentially important therapeutic agents for the treatment of both hyperglycemia and hypoglycemia. This is exemplified by the antidiabetic sulfonylureas, which specifically inhibit K_{ATP} channel function and are widely used in the treatment of type 2 (non-insulin-dependent) diabetes (3). The sulfonylureas are believed to mediate their effects on the K_{ATP} channel via a high affinity sulfonylurea receptor present on the surface of insulin-secreting cells (4). However, the precise relationship between receptor and channel remains unestablished. Results of biochemical studies have demonstrated that the two can be functionally uncoupled by removal of intracellular Mg^{2+} (5) and physically uncoupled by trypsinization (6); recent cloning studies also support the contention that the receptor and channel may be separate entities (7–9).

In addition to the sulfonylureas, a wide variety of other compounds are known to inhibit K_{ATP} channel function and thus stimulate insulin secretion (10). However, these compounds appear to modulate K_{ATP} channel function in a relatively nonspecific manner and at concentrations far in excess of any therapeutic use. Ciclazindol is a monoamine uptake inhibitor (11) originally introduced as an antidepressant (12) but found to be an effective anorectic agent, inducing weight loss in rats (13) and man (14). In the current study, we identified ciclazindol as a novel high-potency hypoglycemic agent that inhibits K_{ATP} channel activity in a structurally and pharmacologically specific manner. Furthermore, we demonstrate that the actions of ciclazindol are mediated via a novel mechanism distinct from that of the sulfonylureas.

Materials and Methods

Preparation of CRI-G1 cells. CRI-G1 insulin-secreting cells were cultured and passaged at 3–4-day intervals as previously described (15). Cells for patch-clamp studies were plated onto 3.5-cm Petri dishes (Falcon 3001) at a density of ~1.5×10^5 cells/dish and were used 2–4 days (inclusive) after plating.

Electrical recording and analysis. We used both cell-free and whole-cell configurations of the patch-clamp recording technique (16). Recording electrodes, when filled with electrolytes, had resistances of 8–12 MΩ for isolated patch experiments and 2–5 MΩ for whole-cell recording. Current recordings were made with an Axopatch 2D or a List EPC-7 patch-clamp amplifier and stored on magnetic tape (Racal 4DS) or digital audio tape (Sony DTC-1000ES)

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ABBREVIATIONS: K_{ATP}, ATP-sensitive potassium; P_o, open probability; I, current responses during drug exposure; TTX, tetrodotoxin; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

for later reproduction of figures and analysis. Single-channel current analysis was determined off-line with the use of PAT 6.2 as previously described (5). Briefly, data segments between 30- and 90-sec duration were filtered at 1.0 kHz with an eight-pole Bessel filter and digitized at 5.0 kHz with a Data Translation 2801A interface. The average channel activity (number of functional channels in a patch × P_o) was determined by measuring the total time spent at each unitary current level and expressed as a proportion of the total time recorded. Changes in the number of functional channels in a patch × P_o as a result of drug application are expressed as a percentage of control.

To obtain whole-cell K_{ATP} channel currents, cells were clamped at a holding potential of −70 mV, and ±10 mV pulses of 200-msec duration were applied at 2-sec intervals. The effects of ciclazindol were quantified by measuring the amplitude of I during drug exposure and comparing them with current responses observed under control conditions immediately preceding application of ciclazindol. The concentration inhibition curves were fitted by nonlinear regression to the following equation: $I/I_c = 1/1 + (a/b)^{n_H}$, where a is the drug concentration, b is the half-maximal inhibitory concentration, and n_H is the Hill coefficient.

The effect of ciclazindol on whole-cell, voltage-activated currents was assessed by applying step depolarizations in 10-mV increments from a holding potential V_h of −60 mV to +40 mV. Macroscopic voltage-activated currents were evoked using the computer program VGEN (14). Thus, inward Na^+ currents were evoked by applying depolarizing voltage pulses of 50-msec duration at a frequency of 1 Hz. Ca^{2+} currents and delayed outward K^+ currents were generated in response to voltage steps of 200-msec duration at frequencies of 0.2 and 1 Hz, respectively. The frequency of stimulation did not affect the amplitude of K^+ or Na^+ currents but slightly reduced the sustained Ca^{2+} current component. Voltage-activated currents were analyzed after signal averaging and leak subtraction using the program VCAN as previously described (17).

All electrophysiological experiments were performed at room temperature (19–24°).

Insulin secretion and [^{3}H]glibenclamide binding studies. For insulin-secretion studies, CRI-G1 cells were plated into six-well plates (Falcon) at a density of 3–6 × 10^5 cells/well and incubated in Dulbecco's modified Eagle's medium supplemented with fetal calf serum 5% (v/v), penicillin (50,000 units l^{-1}), streptomycin (50 mg l^{-1}), and L-glutamine (2 mmol l^{-1}). After 24 hr, the cells were washed four times with 2 ml of buffered salt solution, and ciclazindol or mazindol was applied for 15 min at 37°. Immediately after the addition and at the end of the incubation, 500-μl aliquots were collected and centrifuged briefly, and the supernatants were removed and assayed for insulin immediately or after storage at −20°. Immunoreactive insulin was measured with the use of a double-antibody radioimmunoassay (18), with guinea pig anti-porcine insulin serum (Novo Biolabs, Bagsvaerd, Denmark), nonimmune guinea pig serum and sheep anti-guinea pig serum (Scottish Antibody Production Unit, Carluke, Lanarkshire, UK), and rat insulin (Novo) as standard.

For [^{3}H]glibenclamide binding studies on microsomal membranes, CRI-G1 cells were grown to confluence in 805-cm^2 roller bottles, harvested with trypsin, and centrifuged briefly (150 × g for 3 min). The cellular pellet was resuspended in ice-cold homogenization buffer, homogenized at 0° with a Teflon-pestle glass homogenizer (10 strokes at 500 rpm), sonicated for 10 sec, and then centrifuged for 30 min at 70,000 × g. The resulting pellet was resuspended in storage buffer (50 mM Tris·HCl, pH 7.4), supplemented with protease inhibitors (see Drugs and solutions), and centrifuged again at 70,000 × g. Then, the pellet was resuspended in storage buffer to give a protein concentration of 2–4 mg ml^{-1} and frozen at −70° until assayed. Incubations were performed in 50 mM Tris·HCl buffer containing 0.2 nM [^{3}H]glibenclamide, competitor, and CRI-G1 microsomal membranes (15 μg protein ml^{-1}) in a total volume of 1 ml. Equilibration was for 1 hr and was terminated by the addition of ice-cold buffer. The mixture was immediately filtered through Whatman GF/B filters with a 10-place filtration block. The filters were washed with ice-cold buffer (three times with 4 ml), transferred to scintillation vials, and treated with 10 ml of scintillant (Optifluor). After the filters were allowed to stand overnight, radioactivity was detected by liquid scintillation counting. Nonspecific binding was determined by undertaking parallel incubations in the presence of 1 μM unlabeled glibenclamide. Competition curves were fitted by nonlinear regression to the equation

$$\%\ \text{Uninhibited [}^3\text{H]glibenclamide} = \frac{(100 - NS)}{([X]/IC_{50})^{n_H} + 1} + NS$$

where $[X]$ is the concentration of inhibitor, NS is the percentage of [^{3}H]glibenclamide binding insensitive to inhibitor, and IC_{50} is the inhibitor concentration giving 50% inhibition of inhibitor-sensitive binding. From the calculated IC_{50}, the inhibition constant (K_i) was determined from the following relationship (19): $K_i = IC_{50}/(1 + ([L]/K_d))$, where $[L]$ and K_d are the concentration and dissociation constant of [^{3}H]glibenclamide, respectively.

Drugs and solutions. Chemicals used in this study were glibenclamide, fenfluramine, phentermine, and diethylpropion (Sigma Chemical Co.); ciclazindol and mazindol (Wyeth); and [^{3}H]glibenclamide (50.9 Ci $mmol^{-1}$; DuPont-NEN). Glibenclamide was made up as a 10^{-2} M stock solution in methanol. Ciclazindol, mazindol, phentermine, fenfluramine, and diethylpropion were prepared as 10^{-2} M stock solutions in either distilled water or 0.1 M HCl and appropriate adjustment made to solution pH when added.

For electrophysiological studies, the cells were first washed with solution A, which consisted of 135.0 mM NaCl, 5.0 mM KCl, 1.0 mM $CaCl_2$, 1.0 mM$MgCl_2$, and 10.0 mM HEPES, pH 7.2 with NaOH. For whole-cell voltage-clamp of the K_{ATP} channel currents, the cells were bathed in solution A and the pipette contained solution B, which consisted of 140.0 mM KCl, 2.0 mM $CaCl_2$, 1.0 mM$MgCl_2$, 10.0 mM K-EGTA, and 10.0 mM HEPES, pH 7.2 with KOH (resulting in free Ca^{2+} and Mg^{2+} concentrations of 25 nM and 0.65 mM, respectively). To isolate delayed outward currents, cells were bathed in solution A to which $CdCl_2$ (1 mM) and TTX (300 nM) had been added, and the pipette contained solution C, which consisted of 140.0 mM KCl, 2.0 mM $CaCl_2$, 2.5 mM $MgCl_2$, 2.0 mM ATP, 10.0 mM K-EGTA, and 10.0 mM HEPES, pH 7.2 with KOH. For isolation of Na^+ currents, cells were bathed in solution A that also contained 1 mM $CdCl_2$, and the pipette contained solution D, which consisted of 140.0 mM CsCl, 2.0 mM $CaCl_2$, 1.0 mM $MgCl_2$, 10.0 mM Cs-EGTA, and 10.0 mM HEPES, pH 7.2 with CsOH. To study the effects of ciclazindol on Ca^{2+} currents, cells were bathed in solution A that also contained TTX (300 nM), tetraethylammonium (20 mM), and 4-aminopyridine (4 mM), and the pipette contained solution D.

In experiments with the inside-out patch configuration, the pipette contained solution E, which consisted of 140.0 mM KCl, 1.0 mM $CaCl_2$, 1.0 mM $MgCl_2$, and 10.0 mM HEPES, pH 7.2 with KOH, and the bath contained solution B (with Mg^{2+} present) or solution F, which consisted of 140.0 mM KCl, 10.0 mM K-EDTA, 4.6 mM$CaCl_2$, and 10.0 mM HEPES, pH 7.2 with KOH (resulting in free Ca^{2+} and Mg^{2+} concentrations of ~25 nM and <5 nM, respectively). For outside-out patch recordings, solution B was the pipette solution, and solution E was in the bath.

The buffered salt solution used in the insulin secretion studies had a composition of 136.5 mM NaCl, 5.0 mM KCl, 1.0 mM $MgCl_2$, 1.0 mM $CaCl_2$, 10.0 mM HEPES, and 5.6 mM glucose, pH 7.4 with NaOH, plus 1 g l^{-1} bovine serum albumin (99% pure). The homogenate buffer used in the [^{3}H]glibenclamide binding studies contained 200.0 mM D-mannitol, 65.0 mM sucrose, and 10.0 mM HEPES, pH 7.4 with KOH, supplemented with protease inhibitors, iodoacetamide (1 mmol l^{-1}) soybean trypsin inhibitor (10 μg ml^{-1}), leupeptin (5 μg ml^{-1}), pepstatin-A (0.14 μg ml^{-1}), and phenylmethylsulfonyl fluoride (0.2 mmol l^{-1}).

Statistical analysis. All data in the text are presented as the mean ± standard error of the indicated number of experiments. The statistical significance between experimental groups was assessed with one-way analysis of variance.

Results

Insulin-release studies. When the anorectic drug ciclazindol was applied to the buffered salt solution, it was found to elicit insulin secretion from CRI-G1 cells (20 experiments), with concentrations between 20 nM and 1 μM inducing a significant increase in release ($p < 0.05$), although the increase was always <2-fold (130–170%) over basal secretion (0.80 ± 0.11 pmol/10^6 cells 15 min^{-1}). The maximum response was achieved with 50 nM ciclazindol, which increased release to 170.4 ± 6.4% (five experiments, not shown). In contrast, the maximal response elicited by a maximally effective concentration of tolbutamide (500 μM) under identical conditions was a 335 ± 71.0% (seven experiments) increase in insulin release. The structurally related compound mazindol had no significant ($p > 0.05$) secretagogue action on this cell line over the concentration range tested (10 nM to 100 μM; 18 experiments).

Single-channel studies. Because the control of insulin secretion is believed to be modulated by K_{ATP} channel activity, the effects of ciclazindol were examined electrophysiologically. When applied to outside-out membrane patches excised from CRI-G1 cells, 1 μM ciclazindol reversibly inhibited single K_{ATP} channel activity (Fig. 1a). This inhibition of channel activity was concentration dependent, with 1 μM causing complete inhibition (16 experiments), 100 nM causing a reduction in channel open-state probability (P_o) by 83.6 ± 8.3%, and 10 nM causing a reduction in channel open-state probability (P_o) by 24.2 ± 11.1% (three experiments). The reduction in channel activity was not associated with a change in the single-channel conductance over the range of potentials tested (−50 mV to +50 mV), being 55.4 ± 4.3 pS in control and 54.6 ± 5.1 pS in the presence of 100 nM ciclazindol (six experiments), nor was the the inhibition of P_o induced by ciclazindol voltage dependent over this range. Unlike ciclazindol, mazindol had no effect on K_{ATP} channel activity recorded from outside-out patches (Fig. 1b; four experiments) over the concentration range of 100 nM to 100 μM. Furthermore, three amphetamine-derived anorectic agents [fenfluramine, diethylpropion (Fig. 1c), and phentermine] were also ineffective on K_{ATP} channel activity when applied to outside-out membrane patches at concentrations up to 100 μM (three experiments for each).

Ciclazindol was also found to inhibit K_{ATP} channel activity when applied to the cytoplasmic aspect of inside-out membrane patches. However, under these conditions it was found to produce a weaker channel inhibitory effect than that observed when the drug was applied to the extracellular surface. For example, 10 μM ciclazindol produced 85.3 ± 3.1% (four experiments) inhibition of K_{ATP} channel activity (Fig. 2a), which is in contrast to the complete inhibition of channel activity observed when 1 μM ciclazindol was applied to the external surface. This observation infers that ciclazindol might act to inhibit K_{ATP} channel activity via interaction with an extracellular site. In contrast, the sulfonylureas are believed to act at a receptor facing the cytoplasmic surface (18). In an attempt to determine whether ciclazindol acts to

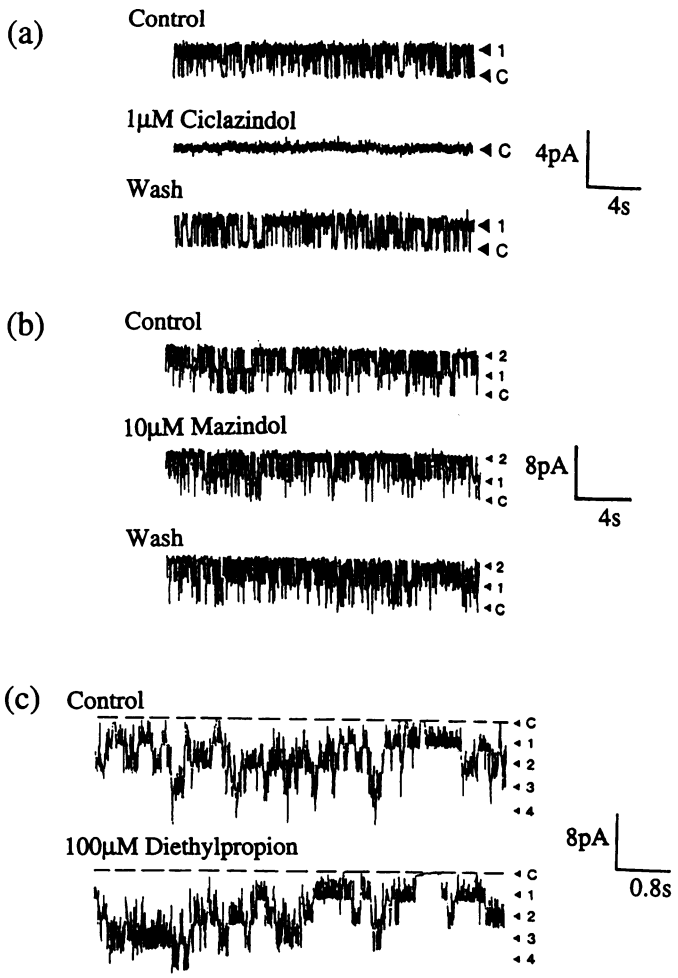

Fig. 1. Effects of ciclazindol and other anorectic agents on K_{ATP} channel currents recorded from outside-out patches isolated from CRI-G1 cells. a, Application of 1 μM ciclazindol to the bath resulted in the complete and reversible abolition of channel activity at a holding potential of +40 mV. The values of P_o were as follows: control, 0.82; 1 μM ciclazindol, 0.00; and wash, 0.80. b, Application of 10 μM mazindol produced no effect on channel activity at a holding potential of +50 mV. The values of P_o were as follows: control, 0.69; 10 μM mazindol, 0.72; and wash, 0.71. c, Application of 100 μM diethylpropion had no effect on channel activity at −50 mV (wash not shown). The values of P_o were as follows: control, 0.25; and 100 μM diethylpropion, 0.23. Drugs were applied for at least 30 sec.

inhibit K_{ATP} channel activity in a manner distinct from that of the sulfonylureas, the effects of ciclazindol on K_{ATP} channel activity were examined in the presence of intracellular trypsin and in the absence of intracellular Mg^{2+}. Both procedures are known to markedly reduce the ability of the sulfonylureas to inhibit K_{ATP} channel activity (5, 6). However, neither procedure was found to significantly affect the ability of ciclazindol to inhibit channel activity. After K_{ATP} channel rundown and reactivation by the application of 100 μg ml^{-1} trypsin to the intracellular surface, 10 μM ciclazindol was found to produce 81.2 + 3.2% (four experiments) inhibition of K_{ATP} channel activity (Fig. 2b), whereas in the absence of intracellular Mg^{2+}, 10 μM ciclazindol inhibited K_{ATP} channel activity by 92.2 ± 3.3% (four experiments, not shown).

Whole-cell studies. To quantify more fully the effects of ciclazindol on K_{ATP} channel activity, the whole-cell recording configuration was used with the twin-pulse protocol de-

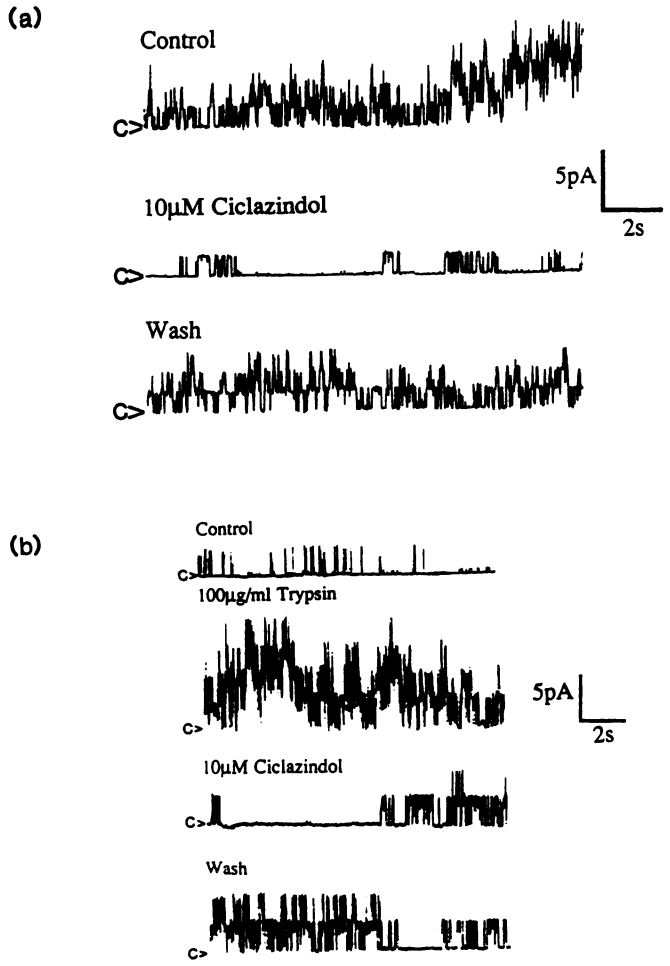

Fig. 2. The effects of ciclazindol when applied to the intracellular surface of excised inside-out patches. a, Single K_{ATP} channel currents recorded at +40 mV. Application of 10 μM ciclazindol produced a marked reversible inhibition of channel activity. The values of P_o were as follows: control, 0.37; 10 μM ciclazindol, 0.02; and wash, 0.23. b, Single-channel recording of K_{ATP} channel activity at +50 mV. Application of 100 μg ml^{-1} trypsin induced a large increase in K_{ATP} channel activity. After trypsinization of rundown patches, the application of 10 μM ciclazindol produced a large and reversible inhibition of K_{ATP} channel activity. The values of P_o were as follows: control, 0.03; 100 μg ml^{-1} trypsin, 0.41; 10 μM ciclazindol, 0.04; and wash, 0.27.

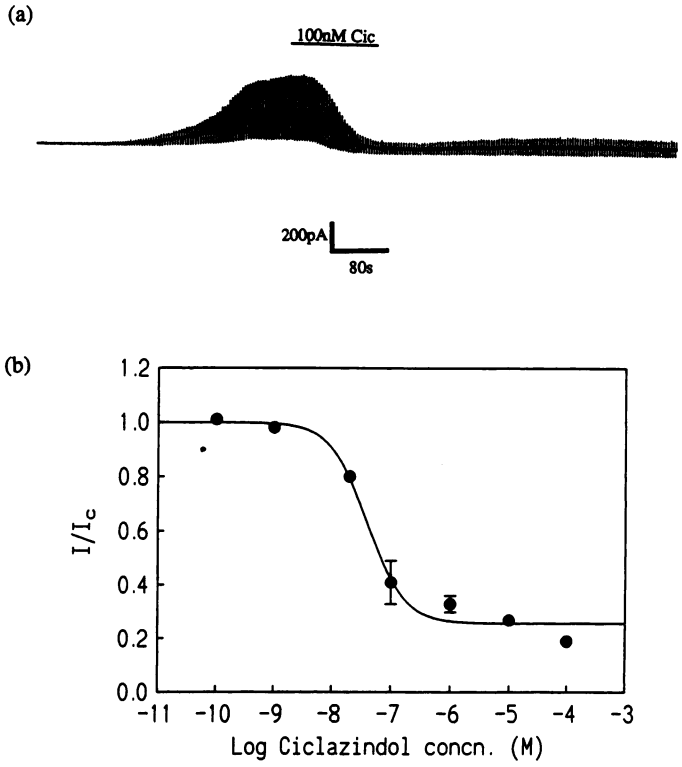

Fig. 3. The effect of ciclazindol (*Cic*) on whole-cell K_{ATP} channel currents. a, The cell membrane was clamped at −70 mV, and ±10 mV pulses were applied for 200 msec every 2 sec. Application of 100 nM ciclazindol to the bath solution was associated with a poorly reversible inhibition of K_{ATP} channel currents (as denoted by the decrease in size of the *vertical lines*) with an associated shift in the holding current (denoted by the *horizontal line*). b, Concentration-inhibition curve for ciclazindol. Data are presented as fraction of the control current taken immediately before the addition of ciclazindol. All points are the mean of three to six separate experiments. *Vertical lines*, standard error; where no line is apparent, the standard error was smaller than the associated symbol. The curve was obtained by nonlinear regression.

scribed in Materials and Methods (Fig. 3). Under these conditions, ciclazindol produced a potent, poorly reversible inhibition of K_{ATP} channel current. From the concentration-inhibition curve, ciclazindol was found to have a half-maximal inhibitory concentration of 40.01 ± 0.60 nM (20 experiments) with an associated Hill coefficient of 1.3 ± 0.3.

To determine whether ciclazindol, like the sulfonylureas, exhibits specificity of action for K_{ATP} channels, the effects of 10 μM ciclazindol were examined on voltage-activated currents in CRI-G1 cells. Voltage-dependent Ca^{2+} currents exhibited a small degree of rundown with repetitive stimulation, even at low frequencies, but this was <15% over a 20-min recording period. The inward Ca^{2+} currents were not significantly affected by the application of ciclazindol to the bath, resulting in a mean inhibition of 3.3 ± 1.4% ($p > 0.1$; three experiments), and there was no effect on the voltage at which the inward currents activated nor the potential at which the maximal inward current was evoked (Fig. 4A).

Under conditions where both Na^+ and Ca^{2+} entries were blocked, ciclazindol had no significant effect on the magnitude of the delayed outward K^+ current (Fig. 4B) or on the time to peak of the currents and their activation threshold (−10–0 mV; five experiments). The amplitude and time to peak of the voltage-activated inward Na^+ currents were not affected significantly by ciclazindol (Fig. 4C). The peak inward Na^+ current ranged from 180–507 pA in the absence of ciclazindol to 155–450 pA in its presence (four experiments), representing <5% inhibition ($p > 0.1$). Furthermore, no change was observed in either the activation threshold potential (−20 mV) or the test potential at which the peak inward Na^+ current was observed (0–10 mV; four experiments).

Ligand binding studies. The results presented above suggest that ciclazindol inhibits K_{ATP} channel activity by a mechanism different than that of the sulfonylureas. To verify this, we examined the ability of ciclazindol to displace [^{3}H]glibenclamide from its binding sites on microsomal membranes of CRI-G1 cells. The K_d calculated for displacement of [^{3}H]glibenclamide by unlabeled glibenclamide in these membranes was 0.91 nM (three experiments). Ciclazindol, over the concentration range tested (10 nM to 100 μM) produced no displacement of [^{3}H]glibenclamide from these membranes (three experiments; Fig. 5).

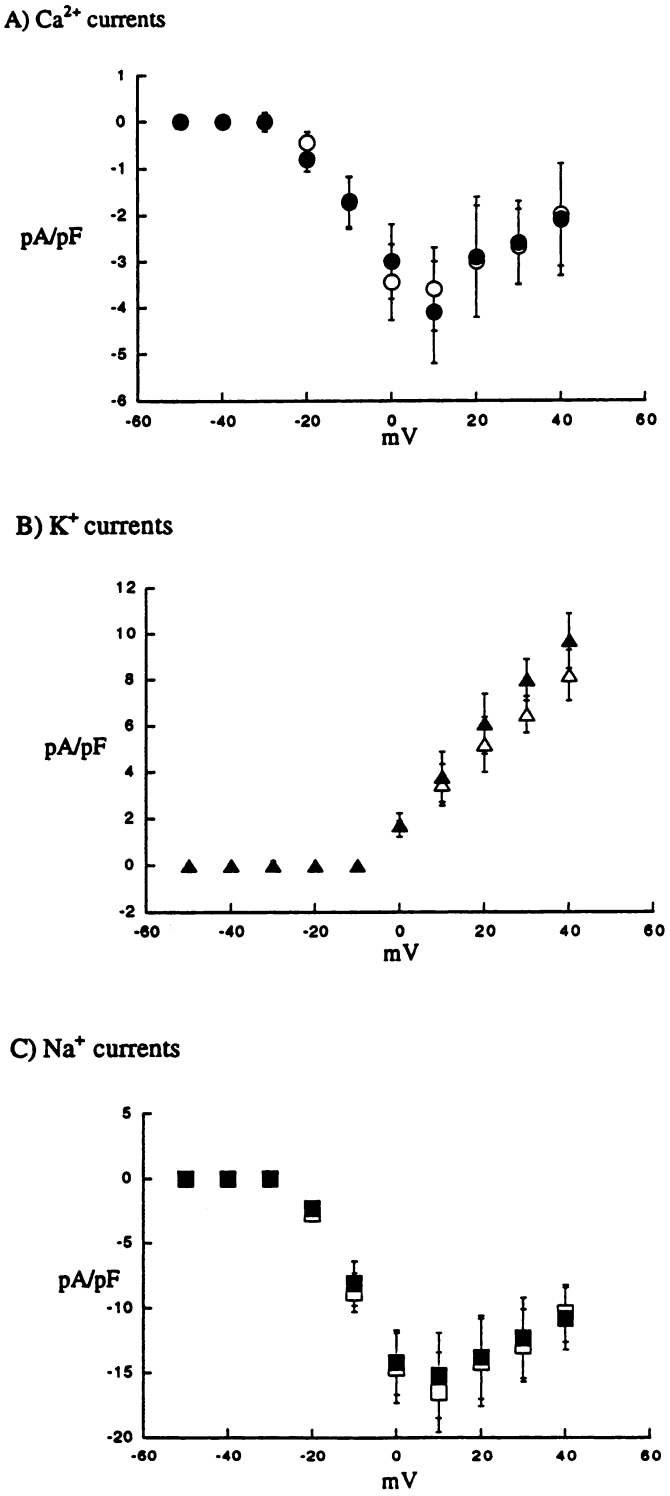

Fig. 4. The lack of effect of 10 μM ciclazindol on whole-cell voltage-activated currents. A, Current-voltage relationship for the Ca^{2+} current in which the peak Ca^{2+} current is plotted as a function of pulse potential. Ciclazindol (●) had no significant effect on the peak current compared with the control (○). The plot represents the mean of three separate experiments. B, Inward Na^+ and Ca^{2+} currents were blocked by the inclusion of TTX and Cd^{2+} in solution A, and outwardly directed voltage-activated K^+ currents were examined. The outward current remaining at the end of the test pulse is plotted against the test potential in the absence (△) or presence (▲) of ciclazindol. Each point represents the mean of at least three separate experiments. C, Current-voltage relationship of the Na^+ current in the absence (□) and presence (■) of 10 μM ciclazindol.

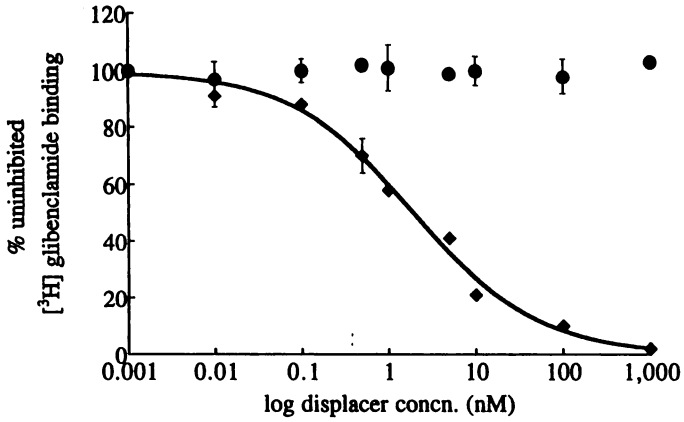

Fig. 5. Displacement curves showing the effects of unlabeled glibenclamide (♦) and ciclazindol (●) on [^{3}H]glibenclamide binding. Each point is the mean of four replicate determinations, with the standard error indicated by the associated error bars. Where no line is apparent, the standard error was smaller than the symbol. Increasing concentrations of unlabeled glibenclamide displaced [^{3}H]glibenclamide with an IC_{50} value of 1.23 ± 0.12 nM. In contrast, ciclazindol failed to affect [^{3}H]glibenclamide binding at concentrations up to 100 μM (not shown).

Discussion

Ciclazindol is an anorectic agent (14) that inhibits monoamine uptake and produces a thermogenic effect in rodents without causing central nervous system stimulation (20). Recent studies indicate that ciclazindol is also a K^+ channel blocker. In an investigation of rat portal vein cell K^+ currents, ciclazindol (1–100 μM) was shown to inhibit the slowly activating and inactivating transient outward current (I_{TO}) and a noninactivating current (I_{KCO}) induced by K^+ channel openers (21). Ciclazindol (100 μM) has also been reported (22) to block whole-cell K^+ currents generated in Chinese hamster ovary cells stably transfected with DNA encoding a mouse voltage-activated K^+ channel (MK-1).

The results presented in this study clearly demonstrate that ciclazindol is an effective inhibitor of K_{ATP} channel activity in the CRI-G1 insulin-secreting cell line. The potency of inhibition of K_{ATP} channel currents by ciclazindol in these insulin-secreting cells is far greater than that reported previously for K^+ channels. Indeed, ciclazindol inhibited K_{ATP} channel currents with a half-maximal inhibition of 40 nM. This compares well with the most potent sulfonylurea, glibenclamide, which half-maximally inhibits K_{ATP} channel currents in this cell line at a concentration of 27 nM (23). Although ciclazindol has a similar potency to glibenclamide for K_{ATP} current inhibition, it appears to be less effective as a secretagogue as the maximum level of insulin secretion attained by ciclazindol was less that that elicited by a maximal effective concentration of either tolbutamide or glibenclamide (23).

Furthermore, the mechanism by which ciclazindol achieves its effects on K_{ATP} channel activity appears to differ from that of the sulfonylureas in several respects. First, ciclazindol appears to inhibit K_{ATP} channel activity more effectively when applied to the extracellular surface. This is in direct contrast to the sulfonylureas, which are believed to inhibit K_{ATP} channel activity by interaction with a specific receptor facing the cytoplasmic surface (24). Second, unlike the sulfonylureas, the effects of ciclazindol are independent of intracellular Mg^{2+} and are retained even after channel

trypsinization. Finally, ciclazindol does not displace high affinity [^{3}H]glibenclamide binding from CRI-G1 cell membrane fragments at concentrations far exceeding that required for maximal inhibition of K_{ATP} channel activity. This agrees with a previous study (21) that demonstrated that ciclazindol had no effect on [^{3}H]glibenclamide binding in porcine brain membrane fragments. These data therefore support the notion that this compound acts at a different site than that of the sulfonylureas.

Mazindol, a structural analogue of ciclazindol, and the amphetamine derivatives phentermine, fenfluramine, and diethylpropion are all anorectic agents and did not inhibit K_{ATP} channel activity in this cell line. Also, mazindol did not act as an insulin secretagogue. Thus, the inhibition of K_{ATP} channel activity by ciclazindol in insulin-secreting cells is specific to this agent and may not appear to be associated with its anorectic actions (13, 14).

Ciclazindol also appears to inhibit K_{ATP} channel currents in this cell line in a pharmacologically specific manner, with no significant effects even at micromolar concentrations on other cation currents present in these cells. This result contrasts with the finding of Noack *et al.* (21), who reported that micromolar concentrations of ciclazindol inhibited various types of voltage-gated K^+ currents in rat portal vein. Thus, it may be inferred that nanomolar concentrations of ciclazindol act specifically to inhibit K_{ATP} channel activity in the pancreatic β cell. This is potentially of clinical importance because it suggests that ciclazindol and structural analogues might achieve hypoglycemic effects at concentrations sufficiently low to prevent deleterious side effects in other tissues.

In summary, we identified the anorectic compound ciclazindol as a novel K_{ATP} channel inhibitor of high potency, although it clearly is not as a good insulin secretagogue as the sulfonylureas in these *in vitro* studies. Furthermore, we clearly demonstrated that this compound acts in a manner distinct from that of the sulfonylureas. Thus, ciclazindol may be a suitable template from which to develop new and important antidiabetic agents. Consequently, it will be instructive to elucidate the mechanism by which ciclazindol achieves its K_{ATP} channel-inhibitory effect.

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