# Effects of $\alpha_2$ -Adrenergic Agonism, Imidazolines, and G-Protein on Insulin Secretion in β Cells

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**It is well known that $\alpha_2$-adrenergic agonism inhibits insulin secretion and stimulates glucagon secretion in both animal and human studies. Recently, $\alpha_2$-adrenergic blockers (DG-5128, MK-912, and SL 84.0418) have been studied as antihyperglycemic agents in human subjects. To clarify the action mechanism(s) of these agents, we investigated the effects of $\alpha_2$ agonists and antagonists ($10^{-10}$ to $10^{-4}$ mol/L) and pretreatment by pertussis toxin (PTX) on glucose-stimulated insulin secretion using the hamster insulinoma cell line HIT-T15. The imidazoline-derivative $\alpha_2$-adrenoceptor agonists clonidine and oxymetazoline at concentrations as low as $10^{-8}$ mol/L significantly inhibited glucose-stimulated insulin secretion by 63% and 65%, respectively ($P < .01$ for both). These inhibitory effects were abolished by 20-hour preincubation of these cells with PTX 100 ng/mL. The imidazoline-derivative $\alpha_2$-adrenoceptor antagonist DG-5128 at a concentration of $10^{-4}$ mol/L doubled insulin secretion with or without pretreatment by PTX ($P < .01$ for both). Furthermore, both clonidine and oxymetazoline at a high concentration of $10^{-4}$ mol/L stimulated insulin secretion with pretreatment of the cells by PTX ($P < .05$ for both). These results indicate that glucose-stimulated insulin secretion is inhibited by $\alpha_2$-adrenoceptor agonists through PTX-sensitive G-protein in HIT-T15 cells. It is also suggested that imidazoline compounds at high concentrations directly stimulate insulin secretion.**


PANCREATIC ISLETS are richly vascularized, and both islet cells and blood vessels are closely associated with a variety of autonomic nerves.[1,2] The sympathetic nervous system plays an important role in the regulation of pancreatic hormonal secretion through the release of catecholamines.[2] We[3-7] and others[8] have shown that its predominant effect is mediated through $\alpha_2$-adrenergic receptors on the inhibition of insulin[3,4,8] and stimulation of glucagon secretion[5-7] in experimental animals and in human subjects.[8] Recently, oral $\alpha_2$-adrenergic blockers have been investigated as antihyperglycemic agents in healthy subjects and non–insulin-dependent diabetic patients, such as midaglizole (DG-5128),[9] MK-912,[10] and SL 84.0418.[11] However, many $\alpha_2$-adrenoceptor agonists (eg, clonidine and oxymetazoline) and antagonists (eg, efaroxan, DG-5128, and SL 84.0418) are imidazoline compounds (Fig 1), as well as the nonselective α-adrenergic blocker, phentolamine. Phentolamine,[12-14] efaroxan,[15,16] and DG-5128,[17] all imidazoline compounds, have been reported to stimulate insulin secretion through closure of ATP-sensitive potassium channels ($K_{ATP}$ channels) independently of $\alpha_2$-adrenoceptor blockade, and imidazoline binding sites on pancreatic β cells have been reported.[18-20]

Furthermore, Seaquist et al[21] have reported that inhibition of insulin secretion by epinephrine and somatostatin is mediated through pertussis toxin (PTX)-sensitive G-protein in rat islets and HIT-T15 cells. The present study was therefore designed to clarify the mechanism(s) of action of imidazoline-derivative $\alpha_2$-adrenoceptor agonists and antagonists on glucose-stimulated insulin secretion, using the hamster insulinoma cell line HIT-T15 with or without pretreatment by PTX.

## MATERIALS AND METHODS

### *Chemicals*

The following drugs were used in the study: clonidine hydrochloride (Tokyo Kasei Kogyo, Tokyo, Japan), oxymetazoline hydrochloride (Sigma, St Louis, MO), and DG-5128 (Daiichi Pharmaceutical, Tokyo, Japan).

### *Cell Culture*

HIT-T15 cells were cultured in RPMI 1640 medium (glucose concentration, 11 mmol/L) supplemented with 10% fetal calf serum, 100 U/mL penicillin, and 0.1 mg/mL streptomycin (Gibco, Grand Island, NY) at 37°C in a humidified atmosphere of 5% $CO_2$ and 95% air. Cells used for experiments were from passages 77 to 81. The cells were plated on a 24-well plate at $2.5 \times 10^5$ cells per well and cultured for 72 hours either with or without a final 20-hour pretreatment with PTX 100 ng/mL.

### *Insulin Secretion Experiments*

HIT-T15 cells from these passages have a high insulin output even without glucose, and the glucose concentration–insulin secretion curve is shifted to the left; insulin secretion at 3 mmol/L glucose was about 80% of that at 5 mmol/L, and it almost plateaued at glucose concentrations of 5, 8, 16.7, and 20 mmol/L. Thus, we used glucose concentrations of 0 mmol/L for basal and 3 mmol/L for stimulation. The grown cells were preincubated at 37°C for 60 minutes in Krebs-Ringer-bicarbonate HEPES buffer containing no glucose for equilibration. Cells were then incubated for 30 minutes with various concentrations of clonidine, oxymetazoline, or DG-5128 (0, $10^{-10}$, $10^{-8}$, $10^{-6}$, and $10^{-4}$ mol/L) with 3 mmol/L glucose.

### *Hormone Measurements*

Insulin levels were measured with a commercially available kit (Eiken, Tokyo, Japan) based on a radioimmunoassay as described previously.[5-7,22]

### *Statistical Analysis*

All data are expressed as the mean ± SEM for four independent experiments. Each experiment was performed in duplicate. Statistical

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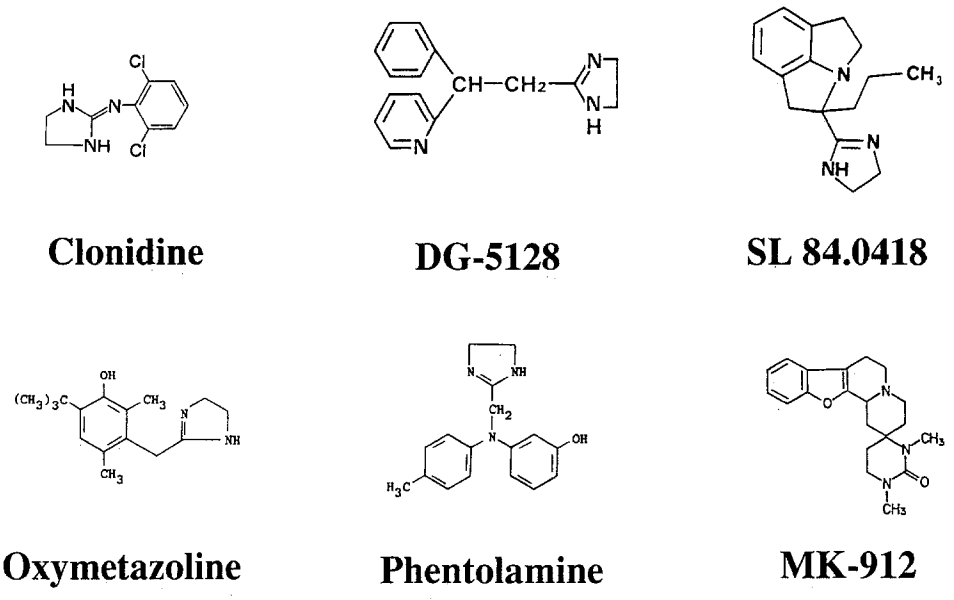


**Fig 1. Chemical structure of $\alpha_2$-adrenergic receptor agonists and antagonists.**

significance of differences was evaluated using two-way ANOVA followed by the Bonferroni/Dunn multiple comparison test (StatView for Macintosh, Version 4.5; Abacus Concepts, Berkeley, CA). Differences were considered statistically significant for $P$ values less than .05.

## RESULTS AND DISCUSSION

### *Effects of $\alpha_2$-Adrenoceptor Agonists Clonidine and Oxymetazoline on Insulin Secretion in HIT-T15 Cells With or Without Pretreatment by PTX*

The imidazoline-derivative $\alpha_2$-adrenoceptor agonists, with clonidine levels as low as $10^{-8}$ mol/L and oxymetazoline as low as $10^{-10}$ mol/L, significantly inhibited insulin secretion ($P < .01$ and $P < .05$ *v* control, respectively) without pretreatment by PTX (Figs 2 and 3). When insulin secretion data were expressed as the glucose-stimulated increment from the baseline value (0 mmol/L glucose data), insulin secretion was inhibited by 62% to 91% by $10^{-8}$, $10^{-6}$, and $10^{-4}$ mol/L of these drugs ($P < .01$ for all; Table 1).

With pretreatment of the cells by PTX, these inhibitory effects of clonidine and oxymetazoline on insulin secretion were abolished at concentrations of $10^{-8}$ and $10^{-6}$ mol/L (Figs 2 and 3 and Table 1). Rather, these compounds at a concentration of $10^{-4}$ mol/L stimulated insulin secretion with pretreatment by PTX ($P < .05$ for both).

### *Effects of Imidazoline-Derivative $\alpha_2$-Adrenoceptor Antagonist DG-5128 on Insulin Secretion in HIT-T15 Cells With or Without Pretreatment by PTX*

DG-5128 at a concentration of $10^{-4}$ mol/L more than doubled insulin secretion with or without pretreatment by PTX ($P < .01$

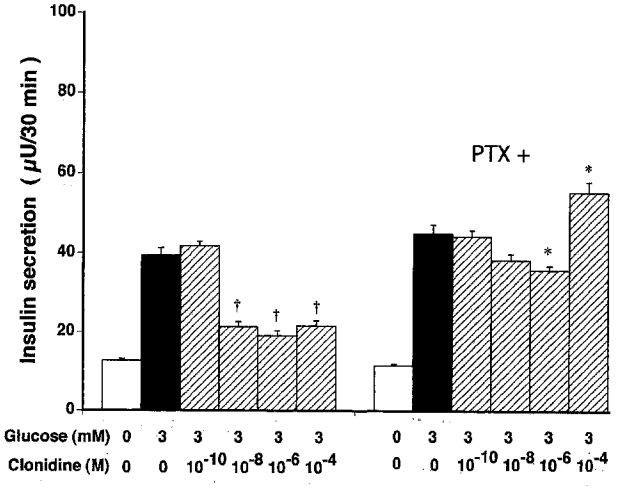


**Fig 2. Effects of the imidazoline-derivative $\alpha_2$-adrenoceptor agonist clonidine on glucose-stimulated insulin secretion in HIT-T15 cells with (right) or without (left) 20-hour pretreatment by PTX 100 ng/mL. Values are the mean ± SEM (n = 4). *$P < .05$ and †$P < .01$ *v* 3 mmol/L glucose (■).**

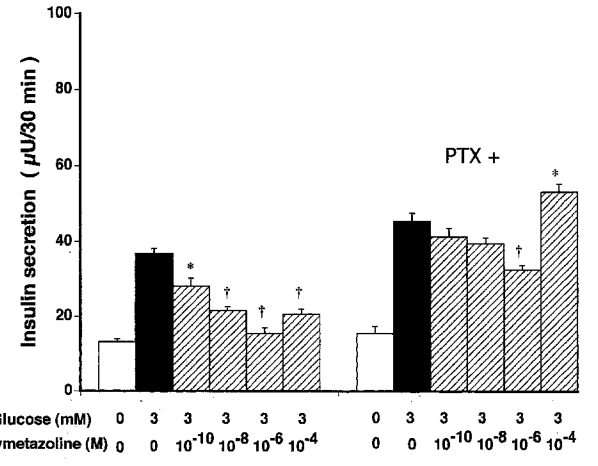


**Fig 3. Effects of the imidazoline-derivative $\alpha_2$-adrenoceptor agonist oxymetazoline on glucose-stimulated insulin secretion in HIT-T15 cells with (right) or without (left) 20-hour pretreatment by PTX 100 ng/mL. Values are the mean ± SEM (n = 4). *$P < .05$ and †$P < .01$ *v* 3 mmol/L glucose (■).**

**Table 1. Effects of Imidazoline-Derivative $\alpha_2$-Adrenoceptor Agonists (clonidine and oxymetazoline) and Antagonist (DG-5128) on 3 mmol/L Glucose-Stimulated Insulin Secretion (μU/30 min) in HIT-T15 Cells With or Without Pretreatment by PTX 100 ng/mL**

| | Concentration (mol/L) | | | | |
|---|---|---|---|---|---|
| | 0 | $10^{-10}$ | $10^{-8}$ | $10^{-6}$ | $10^{-4}$ |
| No. | 12 | 4 | 4 | 4 | 4 |
| No pretreatment | | | | | |
| Clonidine | 23.4 ± 1.1 | 29.0 ± 1.2 | 8.6 ± 1.2† | 6.3 ± 1.2† | 8.8 ± 1.3† |
| Oxymetazoline | | 14.8 ± 2.2† | 8.3 ± 1.2† | 2.2 ± 1.5† | 7.4 ± 1.3† |
| DG-5128 | | 20.6 ± 1.5 | 18.6 ± 1.5 | 25.5 ± 1.4 | 65.7 ± 1.5† |
| PTX pretreatment | | | | | |
| Clonidine | 30.7 ± 1.3 | 32.7 ± 1.7 | 26.8 ± 1.6 | 24.3 ± 1.1 | 43.8 ± 2.6* |
| Oxymetazoline | | 25.8 ± 2.2 | 24.0 ± 1.6 | 17.0 ± 1.2† | 37.7 ± 2.0* |
| DG-5128 | | 23.5 ± 0.7 | 23.1 ± 1.7 | 33.7 ± 3.5 | 73.5 ± 3.5† |

NOTE. Values are the mean ± SEM.
*$P < .05$ and †$P < .01$ *v* 0 mol/L.

for both; Fig 4). When insulin secretion data were expressed as the glucose-stimulated increment (Table 1), stimulation by DG-5128 at $10^{-4}$ mol/L was 139% and 181% with or without pretreatment by PTX, respectively ($P < .01$ for both).

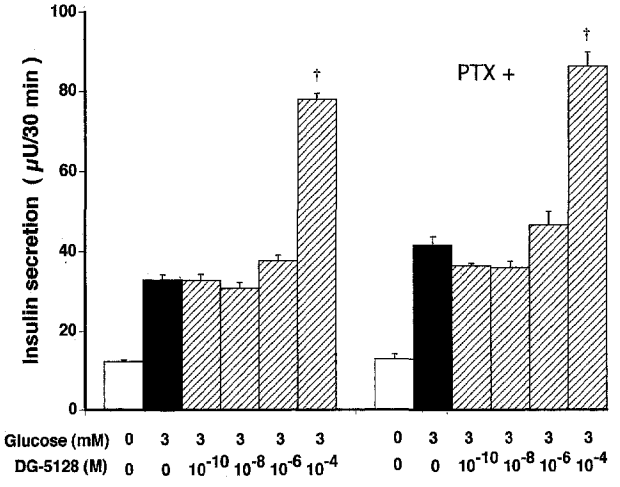


**Fig 4. Effects of the imidazoline-derivative $\alpha_2$-adrenoceptor antagonist DG-5128 on glucose-stimulated insulin secretion in HIT-T15 cells with (right) or without (left) 20-hour pretreatment by PTX 100 ng/mL. Values are the mean ± SEM (n = 4). †$P < .01$ *v* 3 mmol/L glucose (■).**

Recently, there have been several reports concerning imidazoline binding sites on pancreatic β cells.[18-20] Moreover, KU14R, an antagonist of the binding sites, has been developed.[23] However, in the present study, we could not use such a compound and have not verified such binding sites. As for the effector site(s) of $\alpha_2$-adrenoceptors, Seaquist et al[21] have reported multiple G-protein–regulated sites including adenylate cyclase and sites distal to the $K_{ATP}$ channels.

To summarize, we have shown in this study using HIT-T15 cells that imidazoline-derivative $\alpha_2$-adrenoceptor agonists (clonidine and oxymetazoline) inhibited glucose-stimulated insulin secretion at low concentrations of $10^{-10}$ to $10^{-6}$ mol/L. These inhibitory effects were abolished by pretreatment of the cells by PTX. Furthermore, these compounds at a high concentration of $10^{-4}$ mol/L instead stimulated insulin secretion from the cells pretreated by PTX, as well as the imidazoline-derivative $\alpha_2$-adrenoceptor antagonist DG-5128 at $10^{-4}$ mol/L with or without pretreatment. We conclude that clonidine and oxymetazoline have dual effects on glucose-stimulated insulin secretion in HIT-T15 cells: inhibition (predominant effect) mediated through PTX-sensitive G-protein, and stimulation only seen at a high concentration. It is suggested that high concentrations of imidazoline compounds, either as $\alpha_2$-adrenoceptor agonists or antagonists, can stimulate insulin secretion by HIT-T15 cells.

## REFERENCES

1. Woods SC, Porte DJ: The central nervous system, pancreatic hormones, feeding, and obesity. Adv Metab Dis 9:282-312, 1978

2. Miller RE: Pancreatic neuroendocrinology: Peripheral neural mechanisms in the regulation of the islets of Langerhans. Endocr Rev 4:471-494, 1981

3. Nakadate T, Nakaki T, Muraki T, et al: Adrenergic regulation of blood glucose levels: Possible involvement of postsynaptic alpha-2 type adrenergic receptors regulating insulin release. J Pharmacol Exp Ther 215:226-230, 1980

4. Nakaki T, Nakadate T, Kato R: α2-Adrenoceptors modulating insulin release from isolated pancreatic islets. Naunyn Schmiedebergs Arch Pharmacol 313:151-153, 1980

5. Koyama K, Hirose H, Maruyama H, et al: Arachidonic acid metabolites and α2-adrenoceptor–mediated glucagon secretion in rats. Diabetes Res Clin Pract 16:229-232, 1992

6. Hirose H, Maruyama H, Itoh K, et al: Alpha-2 adrenergic agonism stimulates islet glucagon release from perfused rat pancreas: Possible involvement of alpha-2A adrenergic receptor subtype. Acta Endocrinol (Copenh) 127:279-283, 1992

7. Hirose H, Maruyama H, Ito K, et al: Effects of α2- and β-adrenergic agonism on glucagon secretion from perfused pancreata of normal and streptozotocin-induced diabetic rats. Metabolism 42:1072-1076, 1993

8. Metz SA, Halter JB, Robertson RP: Induction of defective insulin secretion and impaired glucose tolerance by clonidine: Selective stimulation of metabolic alpha-adrenergic pathways. Diabetes 27:554-562, 1978

9. Kawazu S, Suzuki M, Negishi K, et al: Studies of midaglizole (DG-5128): A new type of oral hypoglycemic drug in healthy subjects. Diabetes 36:216-220, 1987

10. Ortiz-Alonso FJ, Herman WH, Gertz BJ, et al: Effect of an oral α2-adrenergic blocker (MK-912) on pancreatic islet function in non–insulin-dependent diabetes mellitus. Metabolism 40:1161-1167, 1991

11. Berlin I, Rosenzweig P, Chalon S, et al: Reduction of hyperglycemia after oral glucose load by the new alpha 2-adrenergic receptor antagonist SL 84.0418 in healthy subjects. Clin Pharmacol Ther 55:338-345, 1994

12. Schulz A, Hasselblatt A: An insulin-releasing property of imid-

azoline derivatives is not limited to compounds that block alpha-adrenoceptors. Naunyn Schmiedebergs Arch Pharmacol 340:321-327, 1989

13. Plant TD, Henquin JC: Phentolamine and yohimbine inhibit ATP-sensitive $K^+$ channels in mouse pancreatic beta-cells. Br J Pharmacol 101:115-120, 1990

14. Henquin JC, Charles S, Nenquin M, et al: Diazoxide and D600 inhibition of insulin release: Distinct mechanisms explain the specificity for different stimuli. Diabetes 31:776-783, 1982

15. Chan SLF, Morgan NG: Stimulation of insulin secretion by efaroxan may involve interaction with potassium channels. Eur J Pharmacol 176:97-101, 1990

16. Chan SLF, Dunne MJ, Stillings MR, et al: The α2-adrenoceptor antagonist efaroxan modulates $K^+_{ATP}$ channels in insulin secreting cells. Eur J Pharmacol 204:41-48, 1991

17. Chan SLF, Stillings MR, Morgan NG: Mechanisms involved in stimulation of insulin secretion by the hypoglycaemic alpha-adrenergic antagonist, DG-5128. Biochem Biophys Res Commun 176:1545-1551, 1991

18. Remaury A, Herve P: The insulin-secreting cell line, RINm5F, expresses an alpha-2D adrenoceptor and nonadrenergic idazoxan-binding sites. J Pharmacol Exp Ther 260:417-426, 1992

19. Chan SL, Brown CA, Scarpello KE, et al: Pancreatic beta-cells express an imidazoline binding site that is distinct from I1 and I2 sites. Ann NY Acad Sci 763:153-156, 1995

20. Morgan NG, Chan SL, Brown CA, et al: Characterization of the imidazoline binding site involved in regulation of insulin secretion. Ann NY Acad Sci 763:361-373, 1995

21. Seaquist ER, Neal AR, Shoger KD, et al: G-proteins and hormonal inhibition of insulin secretion from HIT-T15 cells and isolated rat islets. Diabetes 41:1390-1399, 1992

22. Hirose H, Maruyama H, Seto Y, et al: Effects of D-phenylalanine–derivative hypoglycemic agent A-4166 on pancreatic α- and β-cells: Comparative study with glibenclamide. Pharmacology 50:175-181, 1995

23. Morgan NG, Pallett AL, Mourtada M, et al: Synthesis and characterisation of KU14R, a novel antagonist of the pancreatic islet imidazoline binding site. Diabetologia 39:A121, 1996 (suppl 1, abstr)