ELSEVIER

European Journal of Pharmacology 324 (1997) 233–239

ejp

# Antazoline increases insulin secretion and improves glucose tolerance in rats and dogs

Daniel Berdeu [a], Raymond Puech [b], Gérard Ribes [c], Marie-Madeleine Loubatières-Mariani [a], Gyslaine Bertrand [b,*]

[a] *Faculté de Médecine, Laboratoire de Pharmacologie, Institut de Biologie, Boulevard Henri IV, 34060 Montpellier Cedex, France*
[b] *Centre CNRS-INSERM de Pharmacologie Endocrinologie, UPR 9023, Rue de la Cardonille, 34094 Montpellier Cedex 5, France*
[c] *UMR 9921 du CNRS, Montpellier, France*

Received 17 October 1996; accepted 27 January 1997

**Abstract**

In vivo effects of an imidazoline devoid of $\alpha_2$-adrenoceptor antagonistic properties, antazoline, on insulin secretion and glycemia were investigated both in fasted rats and dogs. In both species, antazoline (1.5 mg/kg i.v.) transiently increased insulinemia without affecting basal plasma glucose levels. In contrast, during an i.v. glucose tolerance test, antazoline markedly potentiated insulin release and thus increased the glucose disappearance rate. In rats, during an oral glucose tolerance test, the intragastric administration of antazoline (1.5 mg/kg) clearly enhanced insulin secretion and reduced hyperglycemia. In dogs provided with a venous pancreatico-duodenal bypass, antazoline (0.5 mg/kg i.v.) induced an immediate and transient increase in insulin and somatostatin but not in glucagon pancreatico-duodenal outputs. In conclusion, intravenously and orally administered, the imidazoline antazoline is able to stimulate insulin secretion in vivo and improve glucose tolerance. The imidazoline compounds could therefore have a potential therapeutic relevance as new antihyperglycemic insulinotropic agents.

*Keywords:* Imidazoline; Antazoline; Insulin secretion; Somatostatin secretion; Glucose tolerance; Pancreas; (Rat); (Dog)

## 1. Introduction

It is now widely recognized that imidazoline derivatives provided with $\alpha_2$-adrenoceptor antagonist properties not only exhibit a high affinity for $\alpha_2$-adrenoceptors but also bind to nonadrenergic imidazoline sites in various tissues and species (Michel and Ernsberger, 1992).

In the endocrine pancreas, it was initially reported that imidazoline α-adrenoceptor blockers (such as phentolamine and midaglizole) increased insulin release in vivo (Cerasi et al., 1969; Ahrén and Lundquist, 1985; Kawazu et al., 1987). Since the sympathetic nervous system exerts a negative control on insulin secretion through $\alpha_2$-adrenoceptors (Nakaki et al., 1980), this effect has been attributed to the blockade of an inhibitory adrenergic tone on pancreatic B cells. However, several in vitro studies have clearly demonstrated that the ability of imidazoline compounds to stimulate insulin secretion was specifically related to their structure rather than to their $\alpha_2$-adrenoceptor antagonist action (Schulz and Hasselblatt, 1989; Chan et al., 1991a; Jonas et al., 1992; Berdeu et al., 1994). In addition, the ability of imidazolines to stimulate insulin release has been demonstrated to involve the inhibition of ATP-sensitive $K^+$ channels in pancreatic B cells (Plant and Henquin, 1990; Chan et al., 1991a,b; Jonas et al., 1992). Whereas there is now increasing in vitro evidence that imidazolines can directly stimulate pancreatic B cells by acting on specific imidazoline sites (see Morgan et al., 1995), the effects of these compounds on insulin secretion in vivo and on glycemia remain to be established.

The present work was designed to investigate the in vivo effect of imidazolines on insulin secretion and glucose tolerance. For this purpose, we have tested the effects of antazoline, an imidazoline devoid of $\alpha_2$-adrenoceptor antagonist property, which has been shown in vitro to be a potent imidazoline agonist in increasing insulin release (Schulz and Hasselblatt, 1989; Berdeu et al., 1995). This work was performed both in rats and dogs. In addition, we have investigated in dogs the effects of antazoline on

* Corresponding author. Fax: (33-4) 6754-2432; e-mail: bertrand@ccipe.montp.inserm.fr


*PII* S0014-2999(97)00126-X

secretion of the two other principal pancreatic hormones, glucagon and somatostatin.

## 2. Materials and methods

The experiments were performed in vivo either in male Wistar rats weighing 400–420 g or male mongrel dogs weighing 11–23 kg. Animals were fasted for 16 h before all experimental protocols.

### *2.1. Experiments in rats*

#### *2.1.1. Anesthetized rats*

The animals were anesthetized with an intraperitoneal injection of pentobarbitone sodium (50 mg/kg) and placed on a heated operating surface. Two catheters, filled with heparin-saline to avoid blood clotting, were inserted into the jugular veins, one for injection of test substances and the other for blood samplings. After a 30-min resting period, the test agent solutions (1 ml/kg) were injected. Antazoline (1.5 mg/kg) was diluted in saline; control animals received an equivalent volume of saline alone. For the intravenous glucose tolerance test, glucose (0.5 g/kg) was injected with or without antazoline.

#### *2.1.2. Conscious rats*

The animals were placed individually in cages. Antazoline was administered by intragastric intubation. Blood samples were taken from the tail vessels. Antazoline and glucose were administered together diluted in water; control animals received glucose alone.

In both experiments, blood samples (0.4 ml) were collected in chilled tubes and immediately centrifuged at 4°C for subsequent glucose and insulin determinations.

### *2.2. Experiments in dogs*

#### *2.2.1. Conscious dogs*

Antazoline was administered intravenously (1.5 mg/kg) diluted in 5 ml saline in a jugular vein. For the intravenous glucose tolerance test, each animal received glucose (0.2 g/kg) either alone or with antazoline after a time lag of 2 weeks between the two experiments.

In all experiments, blood samples were taken from the contralateral jugular vein in order to evaluate blood glucose and peripheral plasma insulin levels.

#### *2.2.2. Anesthetized dogs*

Dogs were anesthetized with pentobarbitone (30 mg/kg i.v.). The animals were given heparin intravenously (5 mg/kg) and provided with a venous pancreatico-duodenal bypass. For this purpose, after a median laparotomy, a T-shaped catheter was inserted into the superior pancreatico-duodenal vein just at its exit from the pancreas (Ribes et al., 1983). Blood was sampled from the pancreatico-duodenal vein in order to measure insulin secretion. For this, the clamp was taken off the perpendicular part of the venous T-shaped catheter, and the blood stream towards the portal vein was clamped; blood (5 ml) for pancreatic hormone evaluation was collected in a graduated tube and the time of sampling was measured. Thus, it was possible to determine the venous pancreatico-duodenal blood flow. Thereafter, the blood stream towards the portal vein was re-established. Insulin output rate was determined by multiplying the concentration of the hormone in the plasma by the venous blood flow previously corrected by the hematocrit. Blood glucose concentration was continuously recorded from a peripheral vein with a Technicon autoanalyzer. Femoral arterial blood pressure was recorded with a Ludwig's manometer. After a 60 min equilibration period, antazoline at a dose of 0.5 mg/kg was injected for 1 min into a saphenous vein.

### *2.3. Assays*

Plasma insulin levels were determined by radioimmunoassay using charcoal separation (Herbert et al., 1965), with an anti-porcine insulin antibody (ICN, Biomedicals, Orsay, France) and pure rat or dog insulin (Novo, Copenhagen, Denmark) as the reference standard (the biological activities were 14.2 μU/ng and 22.3 μU/ng, respectively). For glucagon and somatostatin determinations, samples were collected in chilled tubes containing 100 μl of a mixture of EDTA (32 mM) and aprotinin (10 000 kIU/ml) (Antagosan, Hoechst, Puteaux, France). Glucagon concentrations were measured by the method of Unger et al. (1970) using the BR124 glucagon antiserum from the Institut de Biochimie Clinique (Geneva, Switzerland) and porcine glucagon (Novo, Copenhagen, Denmark) as standard. Plasma somatostatin-like immunoreactivity was assayed according to the technique previously described (Ribes et al., 1984) using the 80C antiserum from Dr. R. Unger (Health Science Center, Dallas, TX, USA).

Plasma glucose in rats was determined by the glucose oxidase method (Peridochrom Glucose, Boehringer Mannheim) and blood glucose in dogs was measured with a Technicon autoanalyzer using the potassium ferricyanide procedure.

### *2.4. Drug*

Antazoline hydrochloride was purchased from Sigma.

### *2.5. Data analysis*

The increment in plasma insulin levels was integrated for insulin response. For glucose tolerance tests, two parameters were calculated: the integrated increment in plasma glucose levels and the glucose disappearance rate ($K$) 2–30 min after the glucose load. Data are given as means ± S.E.M. for the indicated number of experiments.

The statistical significance of differences between means was assessed by an unpaired Student's *t*-test, or by a paired Student's *t*-test for the intravenous glucose tolerance test experiments in dogs.

## 3. Results

### *3.1. Effects of intravenous administration of antazoline in anesthetized rats*

The intravenous administration of antazoline at 1.5 mg/kg caused an immediate and transient (2–8 min) increase in plasma insulin levels (Fig. 1, upper panel). The maximum level reached a peak at 2 min: $+50 \pm 8$ μU/ml. No significant effect on plasma glucose levels was recorded (Fig. 1, lower panel).

During the intravenous glucose tolerance test (0.5 g/kg), the increment in plasma glucose concentration reached $+15.7 \pm 1.3$ mM at 2 min and then progressively returned to near-basal values within 60 min (Fig. 2, lower panel). As expected, the rise in glucose levels was associated with a simultaneous increase in insulin secretion; the maximal insulin increment reached $+72 \pm 18$ μU/ml at 2 min (Fig. 2, upper panel). The addition of antazoline at 1.5 mg/kg to the glucose solution markedly enhanced the insulin response; the increment at the peak value was $+139 \pm 15$ μU/ml at 2 min, which is about 2-fold higher than that recorded with control glucose injection. The insulin response was potentiated by antazoline during the first 15 min. The integrated insulin increment was $+1685 \pm 181$ versus $+616 \pm 123$ mU/l with glucose alone ($P < 0.001$). The maximum hyperglycemia was unchanged, but the integrated incremental glucose level during the first 60 min was 27% lower than in controls: $+247.7 \pm 14.1$ versus $+338.5 \pm 23.4$ mM ($P < 0.01$). $K$ for the glucose disappearance rate during the 30 min was increased by antazoline from $2.34 \pm 0.38$ to $3.41 \pm 0.10$%/min ($P < 0.05$).

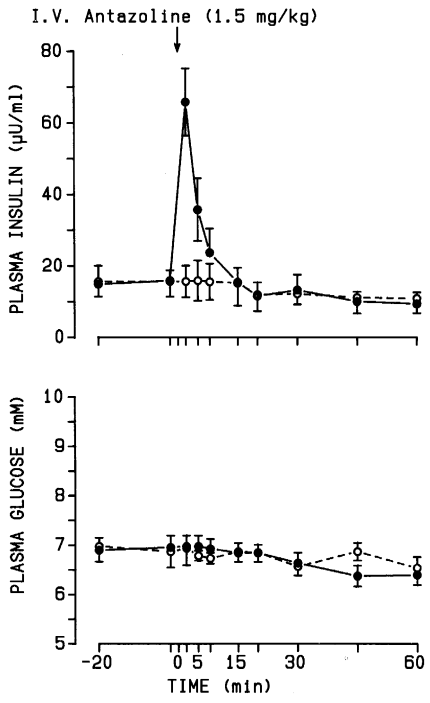


Fig. 1. Effects of an intravenous injection of antazoline (1.5 mg/kg) on plasma insulin and glucose levels in anesthetized fasted rats (●). Control animals received a saline injection (○). Values are means ± S.E.M. of 4 rats.

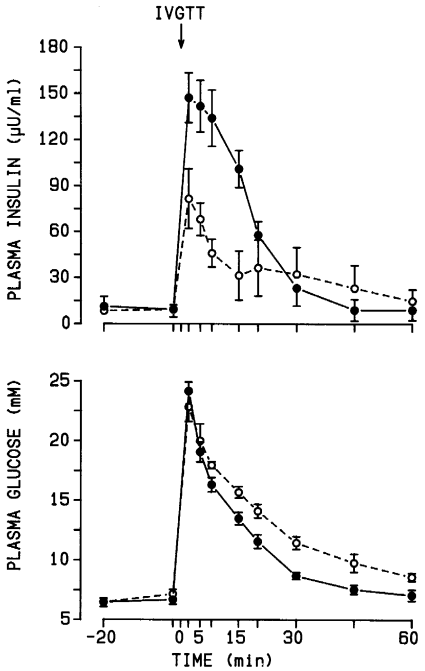


Fig. 2. Effects of an intravenous injection of antazoline (1.5 mg/kg) on increases in plasma insulin and glucose levels in response to an intravenous glucose tolerance test (IVGTT, 0.5 g/kg) in anesthetized fasted rats (●). Control animals received an injection of glucose alone (○). Values are means ± S.E.M. of 5 rats.

### *3.2. Effects of oral administration of antazoline in conscious rats*

The oral glucose tolerance test (1 g/kg) resulted in an increment of plasma glucose and insulin (Fig. 3). When antazoline (1.5 mg/kg) was added to the glucose solution, the insulin response was potentiated and the glucose increment was significantly reduced; thus, the integrated insulin response during the first 30 min was $+1559 \pm 247$ versus $+929 \pm 132$ μU/ml with glucose alone ($P < 0.05$) and the integrated glucose increment during the 120 min was $+312.9 \pm 22.0$ versus $+422.6 \pm 31.4$ mM in controls ($P < 0.05$).

### *3.3. Effects of intravenous administration of antazoline in conscious dogs*

The intravenous injection of antazoline (1.5 mg/kg) provoked an immediate, transient (5 min) increase in

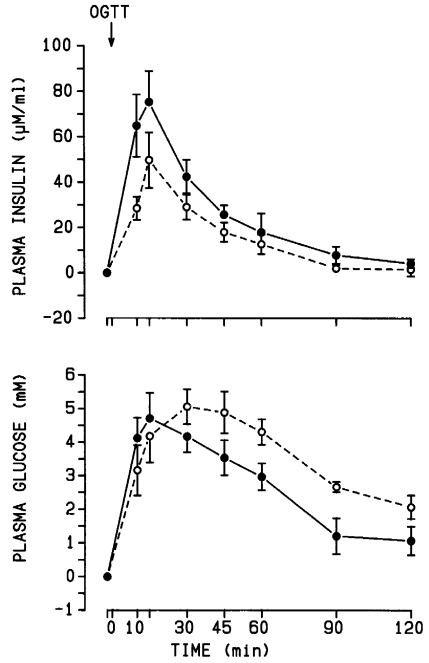


Fig. 3. Effects of an intragastric administration of antazoline (1.5 mg/kg) on increments in plasma insulin and glucose levels in response to an oral glucose tolerance test (OGTT, 1 g/kg) in conscious fasted rats (●). Control animals received glucose alone (○). Values are means ± S.E.M. of 5 rats.

plasma insulin levels (Fig. 4). The maximum increment reached $+17 \pm 2$ μU/ml ($P < 0.001$) at 2 min. No significant effect was recorded on glycemia.

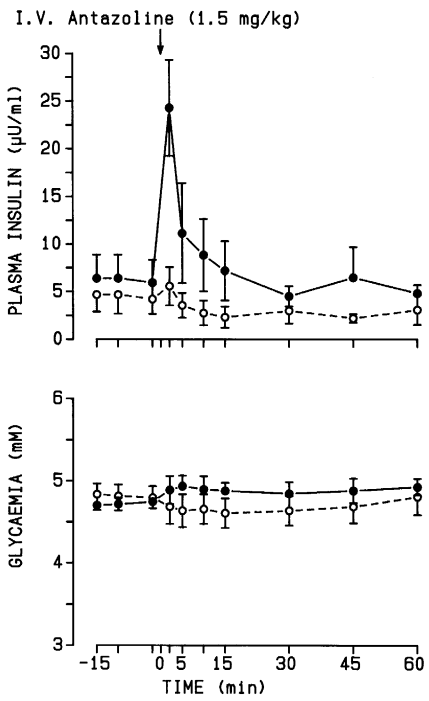


Fig. 4. Effects of an intravenous injection antazoline (1.5 mg/kg) on plasma insulin level and glycemia in conscious fasted dogs (●). Controls animals received a saline injection (○). Values are means ± S.E.M. of 4 dogs.

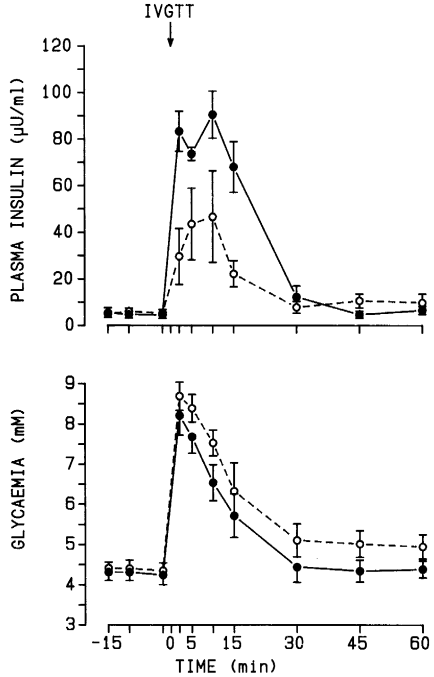


Fig. 5. Effects of an intravenous injection of antazoline (1.5 mg/kg) on increases in plasma insulin and glucose levels in response to an intravenous glucose tolerance test (IVGTT, 0.2 g/kg) in conscious fasted dogs (●). Control animals received glucose alone (○). Values are means ± S.E.M. of 3 dogs.

During an intravenous glucose tolerance test (0.2 g/kg), antazoline (1.5 mg/kg) clearly enhanced the insulin response at least during the first 15 min and consequently reduced hyperglycemia (Fig. 5). The integrated insulin increment was $+1062 \pm 39$ versus $+462 \pm 183$ μU/ml with glucose alone ($P < 0.05$) and the integrated incremental glucose level during the first 15 min was $+38.8 \pm 3.2$ versus $+48.5 \pm 3.3$ mM with controls ($P < 0.05$). $K$ for the glucose disappearance rate during the 30 min was significantly increased by antazoline from $1.99 \pm 0.18$ to $2.22 \pm 0.17\%$/min ($P < 0.05$).

Table 1
Effects of an intravenous injection of antazoline (0.5 mg/kg) on arterial blood pressure and venous pancreatico-duodenal blood flow in anesthetized fasted dogs

| Time (min) | Arterial blood pressure (mmHg) | Blood flow in pancreatico-duodenal vein (ml/min) |
|---|---|---|
| −15 | 145 ± 9 | 11.5 ± 1.8 |
| −10 | 144 ± 9 | 10.9 ± 1.5 |
| −5 | 144 ± 10 | 11.3 ± 1.8 |
| 2 | 146 ± 10 | 11.9 ± 1.6 |
| 5 | 145 ± 11 | 11.3 ± 1.4 |
| 10 | 144 ± 10 | 10.7 ± 1.6 |
| 15 | 145 ± 11 | 10.1 ± 1.3 |
| 30 | 138 ± 19 | 10.0 ± 1.0 |
| 45 | 140 ± 18 | 9.7 ± 1.1 |

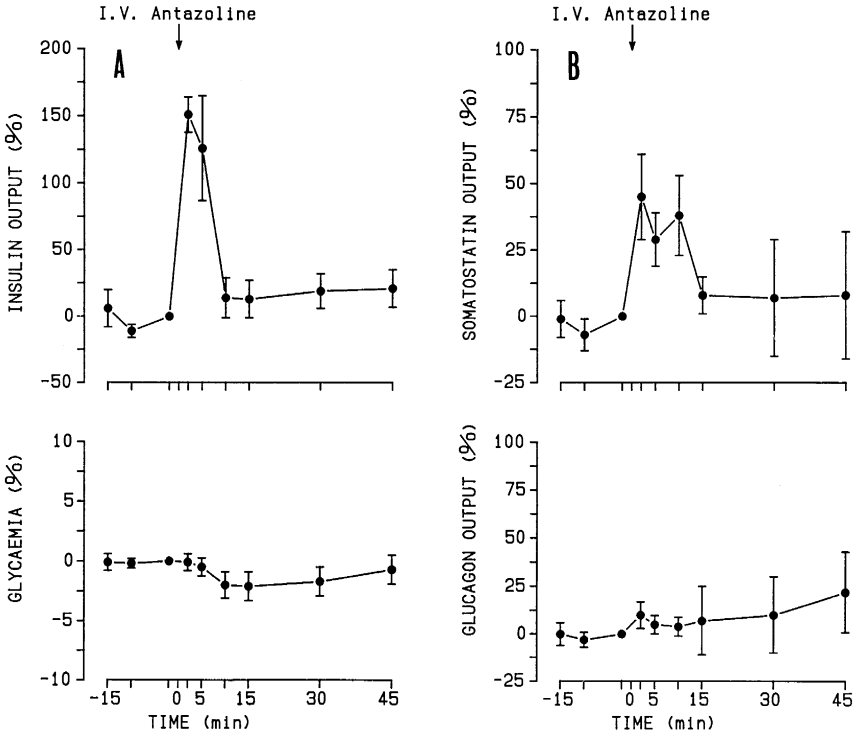


Fig. 6. Effects of antazoline (0.5 mg/kg i.v.) on the pancreaticoduodenal hormone output and glycemia in anesthetized fasted dogs. (A) Effects on the pancreatico-duodenal insulin output and glycemia. (B) Effects on the pancreatico-duodenal somatostatin and glucagon outputs. Results are expressed as changes in relation to the value at time −2 min taken as 100%. The basal values for the insulin output and glycemia were $5.22 \pm 1.16$ mU/min and $5.31 \pm 0.29$ mM, respectively. The basal values for the somatostatin and glucagon outputs were $1031 \pm 199$ pg/min and $1092 \pm 166$ pg/min, respectively. Values are means ± S.E.M. of 4 dogs.

### *3.4. Effects of intravenous administration of antazoline on pancreatico-duodenal hormone outputs and glycemia in anesthetized dogs*

The peripheral intravenous injection of antazoline (0.5 mg/kg) into a saphenous vein induced an immediate and transient (5 min) increase in pancreatico-duodenal insulin output which reached $+150 \pm 13\%$ at 2 min ($P < 0.001$) (Fig. 6A). A slight but not significant reduction in glycemia was recorded ($-2 \pm 1\%$ at min 10). Antazoline also stimulated pancreatico-duodenal somatostatin output: this effect was immediate with a maximum at min 2 ($+45 \pm 16\%$, $P < 0.01$) and remained elevated for 10 min ($+38 \pm 15\%$, $P < 0.05$) (Fig. 6B). In contrast, no significant effect on pancreatico-duodenal glucagon output was observed. Antazoline, at the dose used, did not significantly influence the arterial blood pressure and the pancreatico-duodenal blood flow (Table 1).

## 4. Discussion

This study shows that the imidazoline antazoline induces an increase in insulinemia in vivo and improves glucose tolerance both in rats and dogs. In addition, this imidazoline is able to stimulate somatostatin but not glucagon pancreatico-duodenal release in dogs.

In fasted rats and dogs, antazoline (1.5 mg/kg i.v.) elicited only a slight and transient increase in plasma insulin levels without affecting basal glycemia. In contrast, the same dose of the imidazoline strongly potentiated the insulin response to an intravenous glucose load and this effect was sufficient to improve glucose tolerance which was characterized by a faster decrease of hyperglycemia. In addition, antazoline remained effective on both insulinemia and glycemia after oral administration. Indeed, the intragastric administration of this imidazoline increased insulinemia in response to an oral glucose load and consequently reduced hyperglycemia in fasted rats.

The present study also shows that besides the stimulation of insulin release, antazoline is also able to clearly increase pancreatico-duodenal somatostatin but not glucagon output in dogs. It has been previously demonstrated that the contribution of duodenal somatostatin is marginal in pancreatico-duodenal somatostatin levels (Ahrén et al., 1986) and thus, pancreatico-duodenal somatostatin reflects almost only pancreatic D cell activity (Taborsky and Ensinck, 1984). It is classical that pancreatic B and D cells show similar responses to various stimuli among which increase in glucose or glucagon levels (Weir et al., 1978; Hermansen, 1980); in our experiments, these two parameters were unchanged and do not account for antazoline-induced somatostatin or insulin release. Furthermore, it must be noted that arterial blood

pressure and venous pancreatico-duodenal blood flow were not modified by antazoline administration.

Thus, this in vivo study in two species extends in vitro data and shows that the imidazoline antazoline stimulates insulin secretion and consequently has antihyperglycemic effects with no effect on basal glycemic levels. Furthermore, the imidazoline appears to stimulate somatostatin secretion but is interestingly devoid of glucagonotropic property.

In vivo studies in man and animals have reported that some $\alpha_2$-adrenoceptor antagonists such as phentolamine (Cerasi et al., 1969; Ahrén and Lundquist, 1985) and midaglizole (Kawazu et al., 1987) were able to increase insulin secretion. This observation has been taken as evidence that pancreatic B cells are under an inhibitory sympathetic tone and it has been postulated that this mechanism could contribute to the impaired secretory response in non-insulin-dependent diabetes mellitus (Robertson et al., 1976; Broadstone et al., 1987). However, the blockers effective on insulin secretion such as phentolamine and midaglizole possess an imidazoline ring and have been shown to directly stimulate insulin secretion in vitro independently to an interaction with $\alpha_2$-adrenoceptors (Schulz and Hasselblatt, 1988; Chan et al., 1991b). Nevertheless, another potent imidazoline $\alpha_2$-adrenoceptor antagonist, idazoxan, does not exhibit insulin secretory and antihyperglycemic activities in rats (John et al., 1990), in normal subjects or in patients with non-insulin-dependent diabetes mellitus (Östenson et al., 1988). This discrepancy might be explained by the fact that idazoxan has been shown to be ineffective per se on insulin secretion but to inhibit the secretory effect of imidazolines in vitro (Chan and Morgan, 1990; Berdeu et al., 1995). In this context, antazoline is an imidazoline generally classified as a histamine receptor antagonist which has been reported to exhibit no or weak $\alpha_2$-adrenoceptor antagonist activity in mouse B cells (Schulz and Hasselblatt, 1989; Jonas et al., 1992). More recently, it has also been demonstrated that antazoline did not block $\alpha_2$-adrenergic inhibition of insulin secretion in vivo in the rat (Hiyoshi et al., 1995). In addition, antazoline (like phentolamine) injected i.p. slightly enhanced basal plasma insulin levels whereas the two $\alpha_2$-adrenoceptor antagonists yohimbine and idazoxan were inactive at effective $\alpha_2$-adrenoceptor antagonist concentrations; the authors therefore concluded that antazoline (and phentolamine) induced insulin secretion independently of the adrenoceptors under resting conditions. Thus, since in the present study antazoline clearly exerts insulinotropic and antihyperglycemic actions, an action on imidazoline sites could contribute to the in vivo effects of imidazoline $\alpha_2$-adrenoceptor antagonists. A recent study reports that a new imidazoline derivative (S-21663), which does not bind to $\alpha_2$-adrenoceptors, increased insulin secretion and improved glucose tolerance during an i.v. glucose load in normal and diabetic rats (Wang et al., 1996). However, this compound appears to decrease basal plasma glucose levels in normal rats and the authors reported that it stimulates insulin secretion in vitro independently of the glucose concentration. In contrast, in our study, antazoline does not affect basal glycemia but attenuates only the hyperglycemia induced by i.v. or oral glucose load. The lack of effect of antazoline on basal glycemia could limit the risk of hypoglycemia.

In conclusion, our results show that antazoline, an imidazoline compound devoid of $\alpha_2$-adrenoceptor antagonist property, is able to stimulate in vivo insulin secretion and to improve glucose tolerance without affecting basal plasma glucose levels. The imidazoline compounds could therefore have potential relevance as oral insulin releasing and antihyperglycemic agents in the treatment of non-insulin-dependent diabetes mellitus.

## Acknowledgements

We wish to thank Y. Gueorguieff for expert technical assistance and M. Tournier for illustrations. This work was supported by a grant from Alfediam-Lilly France.

## References

Ahrén, B., Lundquist, I., 1985. Effects of α-adrenoceptor blockade by phentolamine on basal and stimulated insulin secretion in the mouse. Acta Physiol. Scand. 125, 211–217.

Ahrén, B., Paquette, T.L., Taborsky, G.J., 1986. Effect and mechanism of vagal nerve stimulation on somatostatin secretion in dogs. Am. J. Physiol. 250, E212–217.

Berdeu, D., Gross, R., Ribes, G., Loubatières-Mariani, M.M., Bertrand, G., 1994. Effects of imidazolines and derivatives on insulin secretion and vascular resistance in perfused rat pancreas. Eur. J. Pharmacol. 254, 119–125.

Berdeu, D., Gross, R., Puech, R., Loubatières-Mariani, M.M., Bertrand, G., 1995. Evidence for different imidazoline sites on pancreatic B cells and vascular bed in rat. Eur. J. Pharmacol. 275, 91–98.

Broadstone, V.L., Pfeifer, M.A., Bajaj, V., Stagner, J.I., Samols, E., 1987. Alpha-adrenergic blockade improves glucose-potentiated insulin secretion in non-insulin-dependent diabetes mellitus. Diabetes 36, 932–937.

Cerasi, E., Efendic, S., Luft, R., 1969. Role of adrenergic receptors in glucose-induced insulin secretion in man. Lancet ii, 301–302.

Chan, S.L.F., Morgan, N.G., 1990. Stimulation of insulin secretion by efaroxan may involve interaction with potassium channels. Eur. J. Pharmacol. 176, 97–101.

Chan, S.L.F., Dunne, M.J., Stillings, M.R., Morgan, N.G., 1991a. The $\alpha_2$-adrenoceptor antagonist efaroxan modulates $K^+$ATP channels in insulin-secreting cells. Eur. J. Pharmacol. 204, 41–48.

Chan, S.L.F., Stillings, M.R., Morgan, N.G., 1991b. Mechanisms involved in stimulation of insulin secretion by the hypoglycaemic alpha-adrenergic antagonist, DG-5128. Biochem. Biophys. Res. Commun. 176, 1545–1551.

Herbert, V., Law, K.S., Gottlieb, C.W., Bleicher, S.J., 1965. Coated charcoal immunoassay of insulin. J. Clin. Endocrinol. 25, 1375–1384.

Hermansen, K., 1980. Secretion of somatostatin from the normal and diabetic pancreas. Studies in vitro. Diabetologia 19, 492–504.

Hiyoshi, Y., Miura, H., Uemura, K., Endo, H., Ozawa, K., Maeda, N., Tamagawa, T., Iguchi, A., 1995. Effects of imidazoline antagonists of $\alpha_2$-adrenoceptors on endogenous adrenaline-induced inhibition of insulin release. Eur. J. Pharmacol. 294, 117–123.

John, G.W., Doxey, J.C., Walter, D.S., Reid, J.L., 1990. Selective $\alpha_2$-adrenoceptor blockade does not enhance glucose-evoked insulin release. Eur. J. Pharmacol. 187, 531–536.

Jonas, J.C., Plant, T.D., Henquin, J.C., 1992. Imidazoline antagonists of $\alpha_2$-adrenoceptors increase insulin release in vitro by inhibiting ATP-sensitive $K^+$ channels in pancreatic β-cells. Br. J. Pharmacol. 107, 8–14.

Kawazu, S., Suzuki, M., Negishi, K., Watanabe, T., Ishii, J., 1987. Studies of midaglizole (DG-5128). A new type of oral hypoglycaemic drug in healthy subjects. Diabetes 36, 216–220.

Michel, M.C., Ernsberger, P., 1992. Keeping an eye on the I site: imidazoline-preferring receptors. Trends Pharmacol. Sci. 13, 369–370.

Morgan, N.G., Chan, S.L.F., Brown, C.A., Tsoli, E., 1995. Characterization of the imidazoline binding site involved in regulation of insulin secretion. Ann. NY Acad. Sci. 763, 361–373.

Nakaki, T., Nakadate, T., Kato, R., 1980. $\alpha_2$-Adrenoceptors modulating insulin release from isolated pancreatic islets. Naunyn-Schmiedeberg's Arch. Pharmacol. 313, 151–153.

Östenson, C.G., Pignon, J., Doxey, J.C., Efendic, S., 1988. $\alpha_2$-Adrenoceptor blockade does not enhance glucose-induced insulin release in normal subjects or patients with non insulin-dependent diabetes. J. Clin. Endocrinol. Metab. 67, 1054–1059.

Plant, T.D., Henquin, J.C., 1990. Phentolamine and yohimbine inhibit ATP-sensitive $K^+$ channels in mouse pancreatic β-cells. Br. J. Pharmacol. 101, 115–120.

Ribes, G., Blayac, J.P., Loubatières-Mariani, M.M., 1983. Differences between the effects of adrenaline and noradrenaline on insulin secretion in dog. Diabetologia 24, 107–112.

Ribes, G., Trimble, E.R., Blayac, J.P., Wollheim, C.B., Loubatières-Mariani, M.M., 1984. In vivo stimulation of pancreatic hormone secretion by norepinephrine infusion in the dog. Am. J. Physiol. 246, E339–E343.

Robertson, R.P., Halter, J.B., Porte, D., 1976. A role for alpha-adrenergic receptors in abnormal insulin secretion in diabetes mellitus. J. Clin. Invest. 57, 791–795.

Schulz, A., Hasselblatt, A., 1988. Phentolamine, a deceptive tool to investigate sympathetic nervous control of insulin release. Naunyn-Schmiedeberg's Arch. Pharmacol. 337, 637–643.

Schulz, A., Hasselblatt, A., 1989. An insulin-releasing property of imidazoline derivatives is not limited to compounds that block α-adrenoceptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 340, 321–327.

Taborsky, G.J., Ensinck, J.W., 1984. Contribution of the pancreas to circulating somatostatin-like immunoreactivity in the normal dog. J. Clin. Invest. 73, 216–223.

Unger, R.H., Aguilar-Parada, E., Müller, W., Eisentraut, A.M., 1970. Studies of pancreatic alpha cell function in normal and diabetic subjects. J. Clin. Invest. 49, 837–848.

Wang, X., Rondu, F., Laouri, A., Dokhan, R., Marc, S., Touboul, E., Pfeiffer, B., Manechez, D., Renard, P., Guardiola-Lemaitre, B., Godfroid, J.J., Ktorza, A., Penicaud, L., 1996. Effect of S-21663 (PMS 812), an imidazoline derivative, on glucose tolerance and insulin secretion in a rat model of type II diabetes. J. Pharmacol. Exp. Ther. 278, 82–89.

Weir, G.C., Samols, E., Day, J.A., Patel, Y.C., 1978. Glucose and glucagon stimulate the secretion of somatostatin from the perfused canine pancreas. Metabolism (Suppl. 1), 1223–1226.