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Potent antihyperglycaemic property of a new imidazoline derivative S-22068 (PMS 847) in a rat model of NIDDM

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1 Recent data suggest that some imidazoline derivatives can lower plasma glucose in experimental animal models of diabetes. We studied the activity of an imidazoline S-22068, in rat model of non-insulin-dependent diabetes mellitus (NIDDM) produced with a low dose of streptozotocin (35 mg kg^{-1}, i.v.) in the adult.

2 The respective increase over basal value in glucose (ΔG) and insulin (ΔI), and the rate of glucose disappearance (K), were measured during a 30 min intravenous glucose tolerance test. After an intraperitoneal injection of S-22068 (24 mg kg^{-1}), ΔG (mM min^{-1}) was decreased (91.67 ± 5.83 vs 120.5 ± 3.65; $P < 0.001$), whereas K was increased (1.74 ± 0.09 vs 1.18 ± 0.05; $P < 0.001$). Although insulinaemia was increased at time-point 0 of the test, ΔI was unchanged.

3 During oral glucose tolerance tests (OGTT), S-22068 (24 mg kg^{-1}, p.o.) improved glucose tolerance, and its efficiency was potentiated after chronic treatment (15 days). Basal glycaemia was unaffected by the treatment. Under the same conditions, a higher dose of S-22068 (40 mg kg^{-1}) further improved glucose tolerance without causing hypoglycaemia.

4 Binding experiments revealed that S-22068 displays no affinity for either adrenoceptors or the two imidazoline receptors I_1 or I_2.

5 These results demonstrate that S-22068 improves glucose tolerance without causing hypoglycaemia. Thus S-22068 represents a new potential option in the treatment of NIDDM.

Keywords: Glucose tolerance; plasma glucose; insulin secretion; imidazolines; S-22068; NIDDM; imidazoline receptors

Introduction

Non-insulin-dependent diabetes mellitus (NIDDM) is a wide-spread syndrome characterized by fasting and post-prandial hyperglycaemia (Kruszynska & Olefsky, 1996). NIDDM results from an imbalance between insulin sensitivity and insulin secretion in which the ability of insulin to stimulate glucose uptake, mainly into muscle, and inhibit hepatic glucose production is impaired (insulin resistance), while pancreatic B cells are unable to compensate (Leahy, 1990; De Fronzo *et al*., 1992; Beck-Nielsen *et al*., 1992; Consoli, 1990).

Treatments for NIDDM aim to restore normal glucose control by improving insulin secretion or reducing insulin resistance. The compounds available nowadays are mainly sulphonylureas (Gerich, 1989) and biguanides, mostly Metformin (Bailey, 1992). Although they are efficient in most cases, both compounds display adverse side effects. The most common severe complications of sulphonylurea therapy are hypoglycaemia and the so-called 'secondary failure', the major concern with biguanides is the risk of lactic acidosis.

Therefore pharmaceutical industries are still in search of new compounds with better tolerance and longer lasting efficacy. Among all the new molecules tested imidazoline derivates, and closely related compounds, have already proved to be effective in improving glucose tolerance (Kawazu *et al*., 1987; Wang *et al*., 1996; Zaitsev *et al*., 1996; Berdeu *et al*., 1997).

The present study was designed to investigate the *in vivo* effects of a new imidazoline derivative, S-22068, on glucose homeostasis. S-22068 is characterized by a piperazinic system substitued in position 2 by the imidazolinic moiety and in positions 1,4 by an isopropyl (Figure 1). The experiments were performed in rats rendered moderately diabetic by a low dose of streptozotocin (STZ 35 mg kg^{-1}, i.v.).

Methods

Animals and treatments

Three-month-old Wistar rats (Iffa-Credo, L'arbresle, France) weighing about 250 g were used in these experiments. They were housed in wire-bottomed cages and maintained at $21 \pm 2°C$ in a room with a 12 h fixed light-dark schedule. Water and standard laboratory chow (UAR, Villemoisson-sur-orge, France) were freely available.

Moderate diabetes was obtained by i.v. injection of an acute low dose (35 mg kg^{-1}) of STZ dissolved in a citrate buffer under ketamine hydrochloride anaesthesia (75 mg kg^{-1} i.p.; Imalgène, Mérieux, France). These rats were called STZ rats. Glucose homeostasis and insulin secretion were assessed by a glucose tolerance test performed two weeks after STZ injection.

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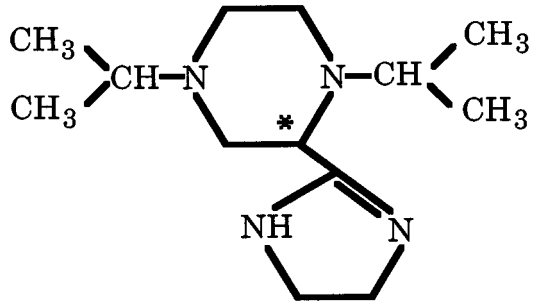

Figure 1 Structure of S-22068 (PMS 847). A racemic mixture of the two eniantiomers was used. *chiral carbon.

Glucose tolerance and insulin secretion tests

For intravenous glucose tolerance test (IVGTT), glucose was dissolved in 0.9% saline and given by the saphenous vein route (0.5 g kg^{-1}) to rats under pentobarbital anaesthesia (75 mg kg^{-1} i.p.; Clin-Midy, France). A 30 min stabilization period preceded the injection of glucose to avoid perturbations due to the induction of anaesthesia. Previous studies showed that after a 30 min recovery period following anaesthesia, glycaemia returns to normal in rats (Penicaud *et al*., 1987). Blood samples were collected sequentially by the tail vessels before and 5, 10, 15, 20 and 30 min after the injection of glucose. They were then centrifuged, and the plasma was separated. Plasma glucose concentration was determined immediately in a 10 μl aliquot and the remainder was kept at $-20^{\circ}C$ until radioimmunoassay for insulin.

For oral glucose tolerance test (OGTT) glucose was given p.o. (2 g kg^{-1}) to conscious rats. Blood samples were collected before and 10, 20, 30, 40, 60, 90 and 120 min after p.o. glucose administration. Treatment of blood samples was identical to that described above.

When we studied the effect of S-22068 on i.v. glucose tolerance and insulin secretion, a single i.p. injection of S-22068 was performed in rats anaesthetized with pentobarbital, 20 min before IVGTT. The drug was administered p.o., 60 min before OGTT.

To study the effect of S-22068 after a 2-week treatment, rats were given a single dose (24 mg kg^{-1}, p.o.) of S-22068, every day, for 2 weeks. Controls received a single daily administration of saline under the same conditions.

Radioligand binding assays

Tissue and membrane preparations were performed as previously described (Wang *et al*., 1996). Membranes were prepared from bovine adrenal medulla glands, rabbit's kidney cortex and calf's frontal cortex. Radioligand binding assays with [^{3}H]-RX821002, [^{3}H]-clonidine, [^{3}H]-idazoxan for determination of specific binding to α_2-adrenergic receptors, I_1- and I_2-imidazoline binding sites respectively, were performed according to the protocol described before (Wang *et al*., 1996). Protein was assessed by a Bradford method (Bradford, 1976).

Safety studies

Central Irwin (1968) test S-22068 was administered per o.s. at 32, 64, 128, 256, 512, 1024 mg/kg to groups of three rats (220–230 g), physiological and behavioural symptoms were noted as follows: mortability, sedation, excitation, aggressiveness, curving of the tail, writhes, convulsions, tremor, exophtalmos, salivation, lacrimation, piloerection, defecation, fear, reactivity to touch, loss of righting reflex, sleep, motor incoordination, muscle tone, stereotypy, catalepsy, graspingness, ptosis, corneal reflex, analgesia, respiration, gait, pupil diameter and rectal temperature.

Cardiovascular studies

Rats ($n=10$) weighed 250 to 300 g. The carotid was catheterized and mean arterial blood pressure and heart rate were measured using a monitor (WECO VT-15, Harvard apparatus, South Natick, U.S.A.) 15 min before and 30 min, 1 and 2 h after the administration of the drug (Schenk *et al*., 1992). S-22068 was administered per o.s. at the dose of 12.5, 25 and 40 mg/kg.

Physioloical well-being of the rats was also assessed by measuring rectal temperature, hematocrit and arterial blood pH, pCO_2 2 h after drug administration.

Analytical methods

Plasma was analysed using a glucose analyser (Beckman Inc, Fullerton, U.S.A.) and plasma immunoreactive insulin concentration was measured with a radioimmunoassay kit with a human standard (Sorin, Biomedica, Antony, France). The lower limit of the assay was 19.5 pmol/l with a coefficient of variation within and between assays of 6%.

Calculations and statistical analysis

Glucose tolerance was measured using two parameters: ΔG, which represents the increase in glycaemia over the baseline integrated over a period of 30 min (IVGTT) or 120 min (OGTT) after the glucose load, and K coefficient which is the rate of glucose disappearance between 5 and 30 min during an IVGTT.

Insulin secretion during an IVGTT (ΔI) was calculated as the incremental plasma insulin values over baseline integrated over 30 min after the glucose load. The insulin response to the glucose load was calculated as the insulinogenic index ($\Delta I/\Delta G$).

Results are expressed as mean $\pm$ s.e.m.. The significance of differences between means was evaluated by Student's test for paired data and the differences between groups were considered significant from $P<0.05$.

Chemicals

[^{3}H]-idazoxan (45 Ci $mmol^{-1}$), [^{3}H]-RX821002 (52 Ci $mmol^{-1}$) were obtained from Amersham Life Science (Buckinghamshire, U.K.), [^{3}H]-clonidine (61.9 Ci/mmol) from NEN (Boston, U.S.A.), yohimbine from Sigma Chemical Co. (St. Louis, U.S.A.), 2-BFI was a gift from Dr Hudson, S-22068 and S-22687 were given by Servier (Courbevoie, France).

Results

Effect of i.p. administration of S-22068 on i.v. glucose tolerance and insulin secretion

STZ-injected rats displayed glucose intolerance and impaired insulin secretion (Figure 2a and b). This situation was stable from 15 days after STZ treatment and remained stable at least 5 weeks after STZ administration.

The insulin concentration at time-point 0 (t_0) (Figure 2b) was similar in the two groups (220 ± 20 vs 230 ± 20 pM; $n = 16$), whereas the t_0 plasma glucose level (Figure 2a) was significantly higher in STZ rats (5.22 ± 0.22 vs 7.73 ± 0.11 mM; $n = 16$; $P < 0.05$).

In STZ rats a single i.p. administration of S-22068 significantly decreased t_0 plasma glucose, but glycaemia stayed within the physiological range (Figure 2a). During the IVGTT test, as reflected in the K, glucose disappearance was improved in animals treated with S-22068 (Table 1). At the end of the test glycaemia returned to the t_0 level (Figure 2a).

In the basal state, as well as at each time-point during the test, the plasma insulin concentration was higher in treated STZ rats than in untreated ones. However, it remained clearly lower than in control rats and, due to the high t_0 value, neither the ΔI nor the $\Delta I/\Delta G$ were significantly different from that of untreated STZ rats (Figure 2b, Table 1).

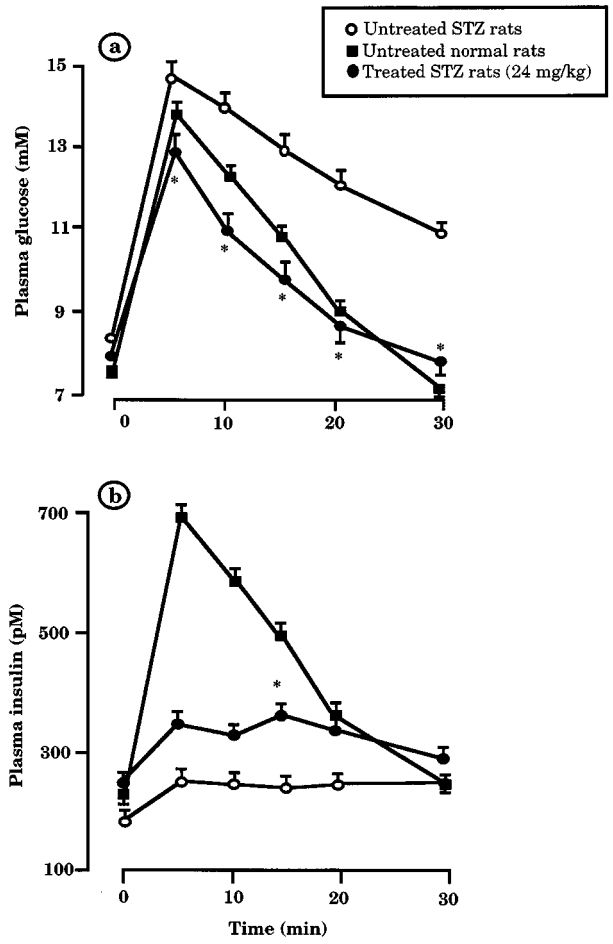

Figure 2 Glucose tolerance and insulin secretion in treated (24 mg kg^{-1}, i.p.) and untreated STZ and normal rats. Values are means $\pm$ s.e.m. ($n = 16$). *$P < 0.05$; significantly different between STZ rats treated with S-22068 and untreated STZ rats.

Effect of p.o. administration of S-22068 on p.o. glucose tolerance

OGTTs were carried out before and after a single p.o. administration of S-22068. In STZ rats, the compound was ineffective on glucose tolerance at the dose of 15 mg kg^{-1} (Figure 3). In contrast, at the dose of 24 mg kg^{-1}, a significant effect on glucose tolerance was observed. Each time-point of the glucose curve was lower in treated than in untreated rats. In these experiments, the ΔG of STZ rats treated with S-22068 was almost one-half that of untreated rats (228 ± 17 mM vs 398 ± 36 mM in untreated rats, $P < 0.001$; $n = 10$).

Effect of p.o. administration of S-22068 on p.o. glucose tolerance after chronic treatment

Chronic treatment for 15 days with 15 mg kg^{-1} day^{-1} of S-22068 had no effect on the parameters of glucose control (Figure 4). A higher dose (24 mg kg^{-1} day^{-1}) of S-22068

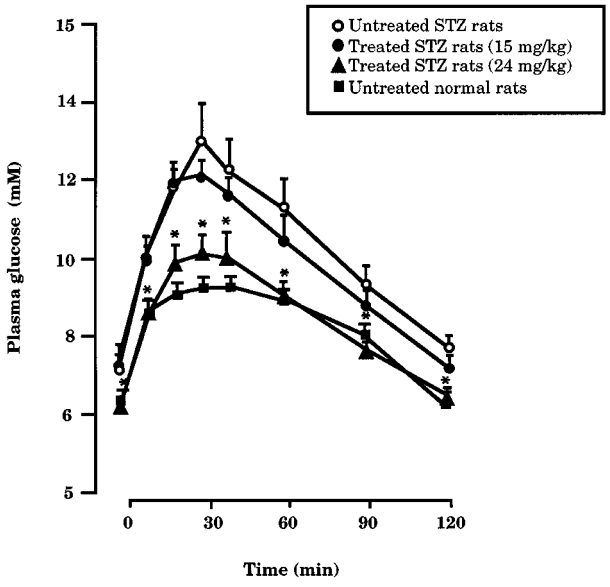

Figure 3 Plasma glucose concentration during an OGTT in STZ rats acutely treated with S-22068, p.o., or not and in untreated normal rats. Values are means $\pm$ s.e.m. ($n = 11$). *$P < 0.05$: significantly different between STZ rats treated with S-22068 and untreated STZ rats.

Table 1 Glucose tolerance (K, ΔG) and insulin response to glucose (ΔI, $\Delta I/\Delta G$) in STZ rats treated or not with a single i.p. dose of S-22068 (24 mg kg^{-1}) and untreated normal rats

	$K \times (-10^{-2})$	ΔG mM	ΔI mM	$\Delta I/\Delta G$ (10^{-9})
Untreated normal rats	2.54 ± 0.09	80.6 ± 3.4	5844 ± 474	74.4 ± 7.0
Untreated STZ rats	1.12 ± 0.06	123.2 ± 4.5	2309 ± 439	20.7 ± 3.5
STZ rats treated with S-22068	1.94 ± 0.08*	100.1 ± 3.6	2171 ± 350	22.1 ± 3.4

*$P < 0.05$, STZ rats treated with S-22068 are statistically significantly different from untreated STZ rats. Values are means $\pm$ s.e.m. ($n = 16$ in all cases), K: rate of glucose disappearance; ΔG: incremental plasma glucose values integrated over 30 min after the glucose load; ΔI: incremental plasma insulin values integrated over 30 min after glucose load; $\Delta I/\Delta G$: insulinogenic index.

greatly improved glucose tolerance (Figure 4). With this dose we obtained a maximal effect of S-22068 since a treatment with a 40 mg kg^{-1} day^{-1} administration of the compound caused no further improvement of plasma glucose parameters.

Binding assays

According to the curves depicted on Figures 5a–c, S-22068 appears devoid of effect on the displacement of the specific ligands of the three receptors studied. S-22068 is a far less specific ligand for α_2-adrenergic receptors than idazoxan or yohimbine (Figure 5a). Its affinity for I_1 or I_2 sites is very weak as well (Figure 5b and c).

Safety data

Preliminary studies showed that S-22068 had no significant central effects as assessed by the Irwin (1968) test. For all parameters, no change was detected from the active pharmacological dose (32 mg kg^{-1}) to the highest dose (1024 mg kg^{-1}) for 24 h. Furthermore, at the various doses, S-22068 did not significantly modify the mean arterial blood pressure and the heart rate 0.5, 1 and 2 h after administration of the drug. Similarly, rectal temperature, hematocrit and arterial blood pH, pCO_2 and pO_2 were not significantly altered.

Discussion

Patients with overt NIDDM display mild basal hyperglycaemia, glucose intolerance and impaired glucose-induced insulin secretion. We have reproduced this feature in Wistar rats treated with streptozotocin at 35 mg kg^{-1}. Therefore this model appears suitable for the study of potential antidiabetic agents, as shown previously (Junod *et al*., 1967; Tancrede *et al*., 1983).

Intraperitoneal administration of S-22068 (24 mg kg^{-1}) caused a clear improvement of i.v. glucose tolerace with a slight increase in plasma insulin levels. The lowering of plasma glucose was not observed in the basal state. Oral administration of the compound in conscious rats confirmed the results on i.p. injections, indicating absorption from the gut. After a single oral administration, S-22068 was not effective at a dose of 15 mg kg^{-1} but was quite effective at the higher dose of 24 mg kg^{-1}. This result may be explained by the fact that molecules of the series to which S-22068 belongs are hardly absorbed through the intestinal barrier (Rondu *et al*., 1996). Thus, we may suppose that 24 mg kg^{-1} is the threshold concentration from which S-22068 can be recovered in the plasma and exerts its antihyperglycaemic effect. The improvement of glucose tolerance was strengthened after a chronic (15 days) oral administration. Under these conditions S-22068 (24 mg kg^{-1}) improved glucose tolerance more potently compared to the acute administration, and significantly improved t_0 plasma glucose levels. These results clearly demonstrate the effectiveness of S-22068 to improve glucose homeostasis. It is noteworthy that the compound improved glucose tolerance while the prevailing insulinaemia was only slightly increased. This can be considered as an advantage,

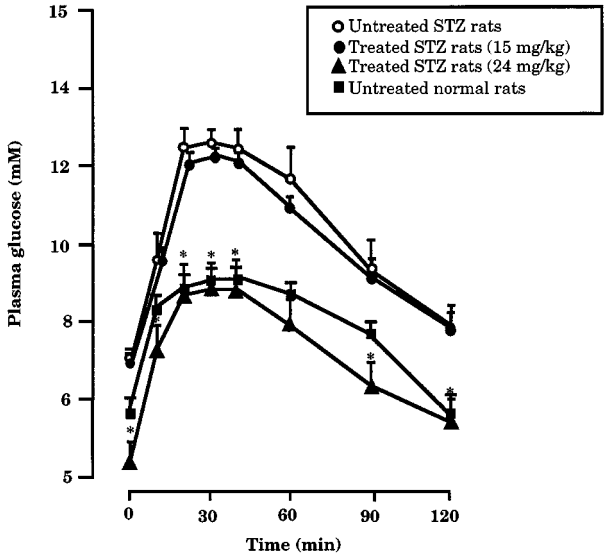

Figure 4 Plasma glucose concentration during an OGTT in STZ rats chronically treated with S-22068, p.o., or not and in untreated normal rats. Values are means ± s.e.m. ($n=6$). *$P<0.05$: significantly different between STZ rats treated with S-22068 and untreated STZ rats.

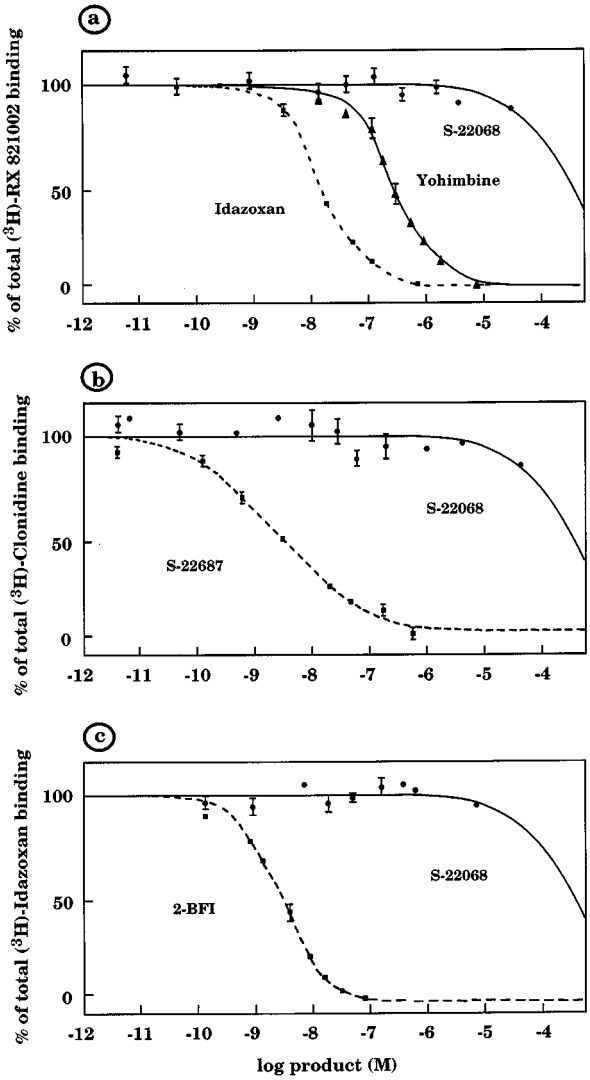

Figure 5 Dose-dependent inhibition of (a): (^{3}H)-RX821002 binding in the presence of idazoxan, yohimbine and S-22068 in calf's frontal cortex; (b): (^{3}H)-clonidine binding in the presence of S-22687-1 and S-22068 on bovine adrenal medulla gland; (c) (^{3}H)-idazoxan binding in the presence of 2-BFI and S-22068 on New Zealand White rabbits renal cortex.

from the viewpoint of treating NIDDM, where sparing islet function seems desirable. Other imidazoline derivatives were efficient on glucose tolerance in diabetic (Wang *et al*., 1996) or normal rats (Berdeu *et al*., 1997). However, their effect was mediated by a marked increase in insulin secretion. The results on safety studies available so far, show an absence of adverse effects of S-22068 on the cardiovascular or nervous systems.

It is now well established, that some imidazolines possess the ability to potentiate nutrient-induced insulin secretion. Since the sympathetic nervous system exerts a negative control on insulin secretion through α_2-adrenoceptors (Miller, 1981), and since overactivity of the autonomic nervous system may contribute to the tonic inhibition of insulin release observed in NIDDM (Robertson *et al*., 1976; Broadstone *et al*., 1987), the improvement of glucose tolerance observed with imidazolines, was first attributed to their blockade of α_2-adrenergic receptors. However, numerous data argue against this conclusion. Several compounds (phentolamine, midaglizole, efaroxan) are able to induce an insulin secretion directly from the perfused pancreas or isolated islets (Schultz & Hasselblatt, 1989; Östenson *et al*., 1989; Chan & Morgan, 1990; Chan *et al*., 1991), while some other α_2-adrenoceptor blockers, are ineffective (yohimbine, rauwolscine) (Schulz & Hasselblatt, 1989; Chan & Morgan, 1990). Moreover, some imidazolines devoid of α_2-adrenergic blockade property (cibenzoline, antazoline) can stimulate insulin secretion, which clearly demonstrates that the insulinotropic capacity of these compounds is not related to their α_2-adrenoceptor blockade and might be due to the presence of the imidazoline moiety in their structure (Bertrand *et al*., 1992; Berdeu *et al*., 1997). Most imidazolines exert their physiological effects through the binding to a receptor. So far, imidazoline receptors have been subclassified in two main subtypes. The first one is represented by the I_1 site preferentially labelled with [^{3}H]-clonidine or its derivative [^{3}H]-*p*-aminoclonidine, and the I_2 site labelled with [^{3}H]-idazoxan (Michel & Ernsberger, 1992). However, addition sites have been indicated in several tissues (Mallard *et al*., 1992; Brown *et al*., 1993; Chan *et al*., 1994; De Vos *et al*., 1994; Molderings & Göthert, 1995; Flamez *et al*., 1997). S-22068 displays neither affinity for classical α_2-adrenoceptors nor for each of the characterized imidazoline binding sites. These data suggest that S-22068 interacts with another receptor distinct from the α_2-adrenoceptor and I_1 and I_2 receptors. Moreover, since we used a racemic mixture there is no definite evidence that S-22068 acts through its binding to a specific receptor.

The mechanism of the antihyperglycaemic effect of S-22068 remains to be determined. Even though this compound possesses obvious insulinotropic properties, the magnitude of insulin secretion cannot fully explain its potent antihyperglycaemic effect. This suggests that S-22068 may also act on glucose metabolism independently of its insulinotropic effect. As mentioned above, sympathetic overactivity and abnormally high catecholamine levels have been reported in NIDDM (Robertson *et al*., 1976; Broadstone *et al*., 1987). Very recently, Youngblood *et al*. (1997) showed that Moxonidine, a centrally activing antihypertensive agent, selective for I_1 imidazoline binding sites (Ernsberger *et al*., 1993) improved oral glucose tolerance in insulin resistant obese Zucker rats. Thus, it cannot be excluded that imidazolines, due to their central nervous system characteristics, may be effective, at least partly, in offsetting insulin resistance.

In summary S-22068 is a potential new antihyperglycaemic agent. It was effective after an acute administration, well tolerated after chronic treatment and did not cause any hypoglycaemia, at least after a 15 day treatment, making it a good candidate for a new generation of antihyperglycaemic agents.

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