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A preliminary, clinical pharmacological assessment of L-659,066, a novel α_2-adrenoceptor antagonist

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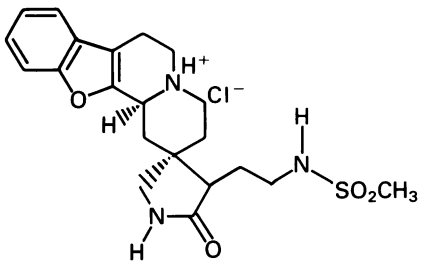
1 The α_2-adrenoceptor antagonist activity of L-659,066 has been investigated in studies of healthy normotensive males to whom doses of up to 8 mg were administered by short intravenous infusion.
2 L-659,066 had no effect on basal levels of glucose or insulin and no significant effect on the plasma glucose and plasma insulin time profiles following an intravenous glucose load.
3 There was a non-significant trend for plasma noradrenaline concentrations to be higher after L-659,066.
4 L-659,066 had no significant effects on mood changes or on physical symptom scores.
5 There were no significant effects on supine blood pressure but there were consistent increases in heart rate both supine (non-significant) and erect ($P < 0.01$).
6 *Ex vivo* platelet aggregation studies confirmed α_2-adrenoceptor antagonist activity with L-659,066 but with an approximately 9-fold lesser potency than yohimbine.
7 While L-659,066 has α_2-adrenoceptor antagonist activity these results suggest that it is unlikely to present a new therapeutic approach for improving insulin release.

Keywords α_2-adrenoceptor antagonism L-659,066 insulin release

Introduction

L-659,066 [2R-trans)-*N*-(2-(1,3,4,7,12b-hexahydro-2′-oxo-spiro (2H-benzofuro,(2,3-a)quinolizine-2,4′-imidazolidin-3′-yl)ethyl methanesulphonamide monohydrochloride] (Figure 1), which has been characterised in animal experiments as a potent and specific antagonist at α_2-adrenoceptors, is a benzofuroquinolizine derivative which only poorly penetrates the blood/brain barrier (Clineschmidt *et al.*, 1988). It is known that the sympathetic nervous system exerts a dual control on the release of insulin: release is enhanced by stimulation of β-adrenoceptors at the pancreatic islet cell and inhibited by stimulation of α-adrenoceptors (Holm, 1983; Porter *et al.*, 1966). Studies in animals suggest that the α-adrenoceptor induced inhibition is mediated through the α_2-adrenoceptor subtype (Langer *et al.*, 1983; Nakadate *et al.*, 1980). Consequently, blockade of the inhibitory α_2-adrenoceptors may facilitate insulin release and thus improve glucose tolerance.

This paper describes a series of three dose finding, safety and tolerability studies designed to investigate aspects of the clinical pharmacology of this novel α_2-adrenoceptor blocking compound, in particular its effect on plasma glucose and insulin levels in response to intravenous glucose loading.



L-659,066

Figure 1 Structure of L-659,066.

Methods

Twelve young, non-diabetic, male volunteers (mean age 25 ± 7 years; mean weight 77 ± 10 kg, mean height 180 ± 6 cm) without any clinical, electrocardiographical, haematological or biochemical evidence of disease gave written, informed consent for their participation in the studies. All protocols had been approved by the Research and Ethical Committee of the Greater Glasgow Health Board. Biochemical and haematological screening was

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repeated prior to and 24 h after each study day as well as 2–4 weeks after completion of the studies.

Study I: Dose finding, safety and tolerability

This study was designed to investigate the safety and tolerability of L-659,066 over a dose range of 0.2–24 mg, administered as a short-term, constant rate, intravenous (i.v.) infusion over 15 min. Three panels of four subjects were randomly selected and all subjects were studied on four occasions, at 1 week intervals, when they received in a double-blind, crossover manner, three sequentially escalating doses of L-659,066 with placebo treatment randomly intercalated. Panel I received 0.2, 0.5 and 1 mg, panel II received 1, 2 and 4 mg and panel III was scheduled to receive 8, 16 and 32 mg total body dose of L-659,066. However, in the first subject who received the highest dose of 32 mg (dose rate: 1.2 mg min^{-1}) the infusion had to be terminated after 5.5 min, i.e. after a total body dose of 12 mg, due to adverse experiences (Subject 11, see Table 1). It was therefore decided to reduce the top dose to be administered to the remaining subjects from 32 to 24 mg. On each study day subjects were monitored for a total of 12 h.

Study II: Effect on plasma glucose and insulin in response to intravenous glucose loading

This double-blind, crossover study compared the effects of 8 mg L-659,066 and placebo on the plasma glucose, insulin and catecholamine responses to an i.v. glucose load. Eight volunteers were studied on two occasions at least 7 days apart when, after an overnight fast, they received a constant rate, intravenous infusion over 15 min of either L-659,066 (8 mg) or placebo. Fifteen minutes after cessation of drug infusion an i.v. glucose load (0.3 g glucose kg^{-1} body weight) over 1 min was administered. Recordings were made until 4 h after dosing when a standard lunch was provided.

Study III: In vitro *platelet aggregation studies*

It is now well established that the adrenergic receptor population in human platelets is predominantly of the α_2 subtype and that adrenaline induced human platelet aggregation is mediated via stimulation of α_2-adrenoceptors (Grant & Scrutton, 1979; Motulsky & Insel, 1982).

In order to obtain preliminary evidence for an α_2-adrenoceptor blocking activity of L-659,066 in *human* tissues the inhibitory effect of 9–12 increasing concentrations of L-659,066 on human platelet aggregation induced by 20 μM adrenaline was studied in platelet-rich plasma (PRP) from five non-smoking male volunteers. This was compared against the inhibitory effect of yohimbine which was chosen as a reference compound. Platelet aggregation was measured as the rate of primary platelet aggregation (mm min^{-1}) by the standard turbidometric method of Born (1962).

Blood pressure and heart rate

Blood pressure and heart rate were measured using a Sentron semiautomatic device. In the safety and tolerability study (Study I) single recordings were carried out every 5 min for the first hour and every 10 min of the second, third and fourth hour post dosing. All subsequent blood pressure and heart rate measurements in Study I and all measurements in Study II were performed as duplicate recordings.

Plasma glucose

Plasma glucose was measured by an enzymatic endpoint assay using hexokinase and glucose-6-phosphate-dehydrogenase (Beckman Instruments, inc., Carlsbad, USA). The day to day precision over a 30 day period is reported to be within 1.7 and 2.2% (Beckman instruction leaflet).

Table 1 Summary of adverse experiences during infusion of L-659,066 Study I and II

Subject	*Study*	*Dose* *Total (mg)*	*Dose* *per BW ($mg\ kg^{-1}$)*	*Adverse experiences*
11	I	12	0.15	Nausea, vomiting, gastrointestinal discomfort
9	I	24	0.30	Restlessness, lightheadedness, perspiration, nausea, gastrointestinal discomfort
2	II	8	0.14	Nausea, vomiting, lower abdominal cramps, perspiration, hot flushes, tingling paraesthesias lower legs and forearms
2	II	12	0.20	Palpitations, lightheadedness, urinary urgency, nausea, abdominal discomfort, hot flushes, tingling paraesthesias lower legs
4	II	12	0.15	Restlessness, perspiration, hot flushes, nausea

Plasma immunoreactive insulin (IRI)

Plasma immunoreactive insulin was measured by radioimmunoassay using cepharose covalently linked to a second antibody assay separation system. This method has a sensitivity of 1.5 mu l^{-1} and a precision of 16% at a level of 7 mu l^{-1}, of 12% at a level of 40 mu l^{-1} and of 10.5% at a level of 30 mu l^{-1}.

Plasma noradrenaline and adrenaline

Plasma noradrenaline and adrenaline were measured by an isotope, radioenzymatic assay based on the method as first described by Peuler & Johnston (1977).

L-659,066 plasma concentrations

L-659,066 concentrations in plasma were analysed by radioimmunoassay with a limit of detection of 1 ng ml^{-1}.

Visual analogue scale (VAS) and physical symptoms score (PSS)

In order to make a preliminary assessment of central nervous system activity of L-659,066 changes in ten different mood states were measured using a 100 mm visual analogue scale. The mood states evaluated were: happy, mellow, calm, anxious, irritable, energetic, sad, fearful, high and drowsy.

In addition subjects also answered a questionnaire for physical symptoms (by graded score 0–3) which are empirically associated with increased adrenergic drive: nausea, urinary frequency, perspiration, palpitations, restlessness, loss of appetite, tremulousness, goose flesh, hot/cold flushes, lacrimation, 'runny' nose, muscle aches and penile erection. Subjects were also asked regularly by the supervising medical staff for side effects.

Statistical evaluation

In Study I, clinical observation of side effects and the maximum placebo corrected changes in haemodynamic, hormonal and glucose changes were chosen to describe safety and tolerability. Due to the small numbers involved in each panel no attempts of a formal statistical analysis were made.

In Study II blood pressure, heart rate and plasma catecholamine time profiles were statistically compared for treatment differences by analysis of covariance (ANCOVA) using baseline heart rate and blood pressure and baseline catecholamines respectively as covariate.

The areas under the plasma glucose and plasma insulin time curves (AUC), the first plasma glucose and insulin response (AIR) and the first order elimination rate constant for glucose elimination (k_{el}) were compared using paired *t*-test.

A *P* value of less than 0.05 was regarded as significant. 95% Bonferroni confidence intervals (CI) were constructed to describe the magnitude of treatment differences.

Results

Study I—Safety and tolerability

L-659,066 at doses exceeding 12 mg total body dose produced a syndrome of adverse experiences comprising nausea and lower gastrointestinal discomfort, associated with restlessness and lightheadedness. Variable features were hot flushes, urinary urgency and tingling paraesthesias in the distal limbs. These were generally of mild to moderate nature. In most cases these adverse experiences started during the final 5 min of the infusion and subsided completely within 45–60 min after its cessation without any long-term sequelae. Correcting for body weight they appeared at doses exceeding 0.14 mg kg^{-1} (range 0.14 to 0.30 mg kg^{-1}) and at infusion rates exceeding 0.5 mg min^{-1} (range 0.5 to 2.7 mg min^{-1}. Table 1 gives a summary of all side effects experienced in both Study I and Study II.

At the higher dose levels, L-659,066 produced slight increases in supine heart rate and blood pressure. There were no consistent changes in either fasting glucose, insulin or plasma catecholamines.

Study II—Glucose challenge

Plasma glucose and insulin The total and baseline corrected area under the plasma glucose and plasma insulin curves were calculated using the trapezoidal rule. The acute glucose and acute insulin responses respectively (AGR, AIR) were defined as the mean of three plasma glucose and insulin levels respectively taken at 3, 4 and 5 min after i.v. glucose loading. The five glucose levels between 10 and 60 min post glucose injection were used to estimate the first order elimination rate constant (k_{el}) for glucose elimination. They were log transformed (natural logarithm) and plotted against time post-glucose administration: k_{el} was then derived as the slope of the linear regression analysis.

L-659,066 had no effect on fasting (i.e. pre-glucose challenge) plasma glucose and insulin levels. Plasma glucose and insulin time profiles after intravenous glucose loading were similar for both treatments (Figure 2). There were no statistically significant treatment differences for either the total or baseline corrected areas under the glucose and insulin curve, the acute glucose or insulin responses or the rate constant for glucose elimination (Table 2).

Plasma catecholamines In order to take account of the between treatment baseline differences and the effect of intravenous glucose injection on supine catecholamine levels (Figure 3) statistical analysis was carried out separately for the pre-glucose challenge (0–30 min protocol time) and post-glucose challenge (30–120 min protocol time) period using the pre-infusion (0 min) and pre-glucose injection level (30 min protocol time) respectively as covariate. Noradrenaline levels post-glucose injection required log transformation to achieve normal distribution. Plasma noradrenaline levels were consistently higher after pre-treatment with L-659,066. However, taking into account the baseline differences and considering the rather large standard deviations this difference was not statistically significant. Supine plasma

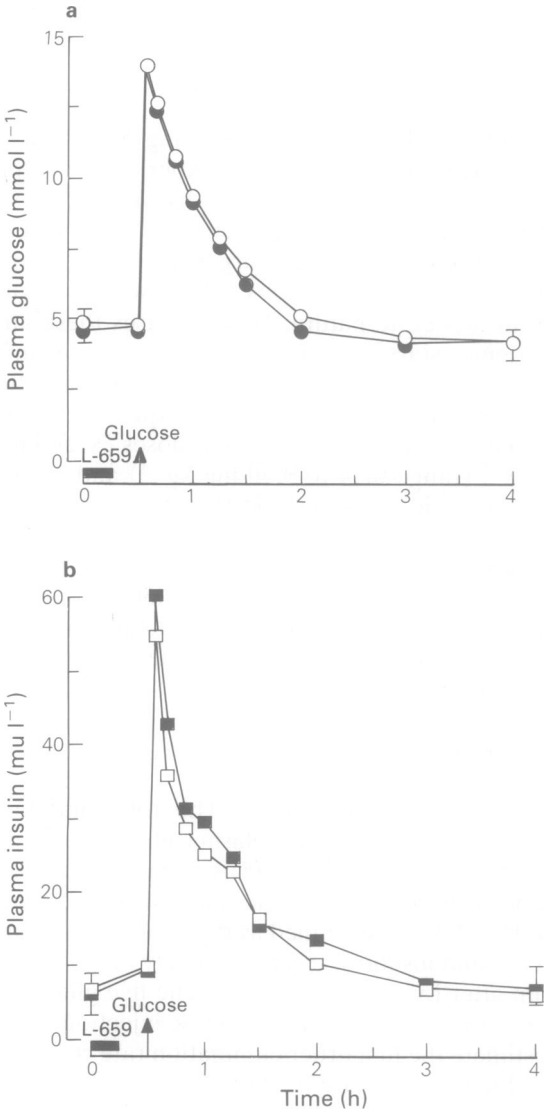

Figure 2 a) Mean plasma glucose-time profiles following L-659,066 (●) and placebo (○) and b) mean plasma insulin-time profiles.

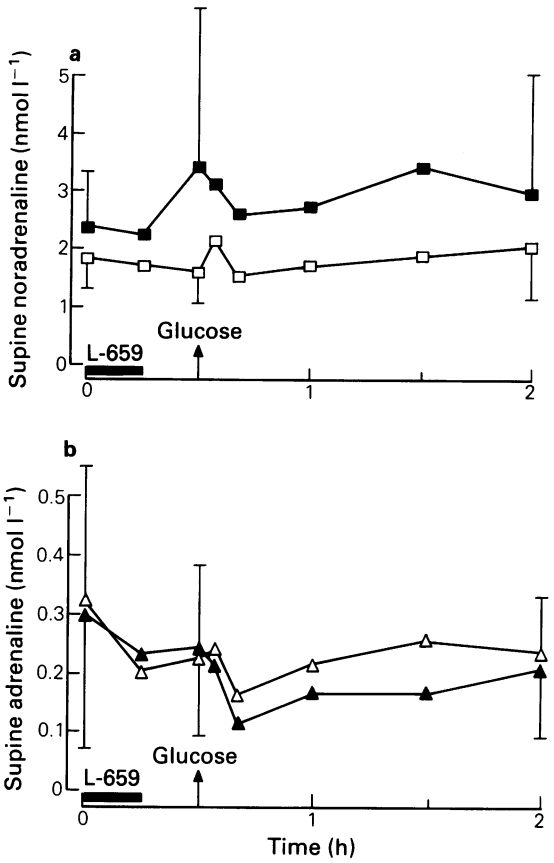

Figure 3 a) Mean supine plasma noradrenaline (L-659,066 (■), placebo (□)) and b) mean supine plasma adrenaline (L-659,066 (▲), placebo (△)).

adrenaline levels were not affected by L-659,066 prior to glucose administration but were significantly lower after i.v. glucose loading (95% CI: −0.13 to −0.01 nmol l^{-1}, $P = 0.05$) (Figure 3).

Visual analogue scale and physical symptoms score L-659,066 had no effect on mood changes or physical

Table 2 Summary of glucose and insulin responses (mean ± s.d.)

	L-659,066	*Placebo*
Glucose		
Acute glucose response (mmol l^{-1})	13.5 ± 1.1	13.9 ± 2.1
Total glucose AUC (mmol l^{-1} h)	20.5 ± 0.9	21.4 ± 2.4
Δ AUC (mmol l^{-1} h)	4.0 ± 3.3	4.6 ± 1.7
k_{el} (min^{-1})	0.0140 ± 0.0029	0.0128 ± 0.0033
Insulin		
Acute insulin response (mu l^{-1})	61.6 ± 31.6	54.7 ± 29.5
Total AUC (mu l^{-1} h)	55.3 ± 23.0	49.0 ± 16.0
Δ AUC (mu l^{-1} h)	22.1 ± 15.7	19.9 ± 11.3

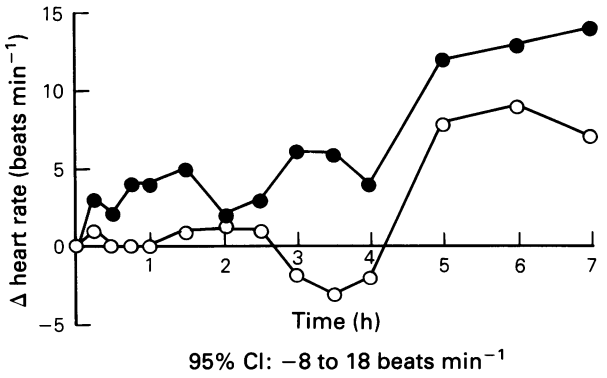

Figure 4 Mean change (from baseline) in supine heart rate (L-659,066 (•), placebo (○)).

symptoms as evaluated by visual analogue scale and physical symptoms score.

Blood pressure and heart rate There were no significant differences between treatments for supine blood pressure and heart rate although there was a slight but consistent increase in heart rate from baseline after administration of L-659,066 which was not observed after placebo (Figure 4) (95% CI: −8 to 18 beats min^{-1}, $P = 0.09$ by ANCOVA). In addition, erect heart rate measured at 120 and 240 min post-dosing was significantly accelerated by L-659,066 (95% CI: 2.7 to 15.9 beats min^{-1}, $P < 0.011$ by ANOVA, treatment mean 95.0 *vs* 86.7 beats min^{-1}). L-659,066 had no significant effect on erect systolic or diastolic blood pressure.

L-659,066 plasma concentrations Five minutes after cessation of the constant rate infusion the mean L-659,066 plasma concentration was 720 ± 144 ng ml^{-1}, range 505–890 ng ml^{-1} (= 1.58 μM ± 0.32, range 1.11–1.96 μM).

Study III—Platelet aggregation studies

The concentrations of L-659,066 and yohimbine required to inhibit *in vitro* platelet aggregation induced by 20 μM adrenaline by 50% (IC_{50}) were calculated (Figure 5 shows the platelet results for a representative subject). L-659,066 suppressed *in vitro* platelet aggregation with an IC_{50} value of 5.38 ± 0.63 μM. The IC_{50} value for yohimbine was 0.58 ± 0.08 μM (Table 3). On average

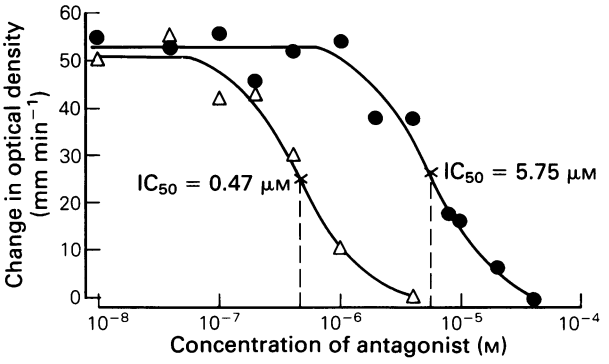

Figure 5 Inhibitory effect of L-659,066 (•) and yohimbine (△) on adrenaline (20 μM) induced human *in vitro* platelet aggregation from a representative subject.

Table 3 Inhibitory effect of L-659,066 and yohimbine on adrenaline (20 μM) induced human *in vitro* platelet aggregation. Summary of IC_{50}-values (μM) and IC_{50}-ratio

Subject	*L-659,066*	*Yohimbine*	*L-659,066/Yohimbine*
1	5.88	0.69	8.5
2	5.89	0.59	10.0
3	4.56	0.57	8.0
4	5.75	0.47	12.2
5	4.84	0.58	8.3
Mean	5.38	0.58	9.4
± s.d.	0.63	0.08	1.7

L-659,066 was thus approximately 9-fold less potent than yohimbine in antagonising human *in vitro* platelet aggregation induced by 20 μM adrenaline.

Discussion

Because of adverse experiences the maximum total body dose was constrained to 8 mg L-659,066 administered as a constant rate intravenous infusion over 15 min. Although several of the differences between placebo and L-659,066 were consistent with α_2-adrenoceptor antagonism (e.g. the slight increases in plasma noradrenaline and in heart rate without a concomitant fall in blood pressure) these differences were very small, did not reach statistical significance and, more importantly, did not suggest any biological significance. Similarly the predominant adverse experiences of lower abdominal discomfort and cramps, associated with nausea, were also consistent with blockade of α_2-adrenoceptors since, in animal experiments, stimulation of intestinal α_2-adrenoceptors has been shown to reduce intestinal motility and colonic propulsion by inhibiting the rate and force of peristaltic contraction (Doherty & Hancock, 1983; Pendleton *et al.*, 1986) and L-659,066 has been shown to antagonise this inhibitory effect (Clineschmidt *et al.*, 1988). The adverse experiences in these present studies, therefore, are consistent with blockade of the inhibitory intestinal α_2-adrenoceptors giving rise to increased intestinal motility and the sensation of abdominal discomfort and nausea.

The *in vitro* assessment of adrenaline induced platelet aggregation also produced results which were consistent with α_2-adrenoceptor antagonism by L-659,066 but with a potency of about 9-fold less than with the 'classical' α_2-adrenoceptor antagonist yohimbine. By extrapolation from the *in vitro* concentrations the peak plasma concentration of 1.58 μM L-659,066 would have been sufficient to cause a slight (5–10%) inhibition of the platelet aggregation induced by 20 μM adrenaline. However, this concentration of adrenaline is 100,000 times higher than the physiological concentration of about 0.2 nM which was measured in this study.

Overall, these findings suggest that the concentrations achieved with 8 mg L-659,066 were sufficient to produce some degree of α_2-adrenoceptor blockade but the failure to demonstrate enhanced insulin release and improved glucose tolerance suggests either that the effect on pancreatic α_2-adrenoceptors was insufficient or that

blockade of pancreatic α_2-adrenoceptors is not directly involved in the regulation of insulin release in response to elevated glucose levels. Studies with idazoxan would support the latter view since i.v. glucose loading failed to produce any significant effect on insulin release and glucose tolerance (Ostenson *et al.*, 1988; Struthers *et al.*, 1985) whereas stimulation of pancreatic α_2-adrenoceptors was able to suppress the release of insulin but in a setting of increased β-adrenoceptor stimulation. In those studies where an enhancement of insulin release has been observed the drugs involved (phentolamine and medaglizone) possess other pharmacological properties such that an insulin mobilising effect may be the result of mechanisms other than α_2-adrenoceptor blockade (Kawazu *et al.*, 1987; Robertson *et al.*, 1976; Yamanaka *et al.*, 1984).

In conclusion the results of these studies (in context with other published reports) would argue against α_2-adrenoceptor blockade with L-659,066 as a new therapeutic approach in non-insulin dependent diabetes mellitus.

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