

S15261 antagonises amylin-induced impaired glucose tolerance

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Abstract Amylin has been postulated to antagonise or inhibit the action of insulin in peripheral rat tissues and thus contribute to, or be responsible for, the development of insulin resistance. We have recently reported that S15261 is a compound capable of increasing insulin sensitivity in ageing insulin resistant rats. In order to assess whether S15261 had any effects on amylin induced insulin resistance we used a model where amylin causes an impairment in glucose tolerance in an acute manner, by means of an intraportal infusion of the hormone in normal rats. We report here that S15261 can antagonise this amylin-induced impaired glucose tolerance.

Key words: Amylin; Insulin resistance; Diabetes; S15261; Portal vein

1. Introduction

Amylin, or islet amyloid polypeptide (IAPP) is the peptide component of amyloid deposits present in the pancreas of non-insulin dependent diabetics and in patients with insulinomas [1–3]. Amylin is present in normal pancreatic β -cells in a variety of species including man, and is co-secreted with insulin, generally in a ratio of 1:20–70 (insulin-to-amylin) [1]. Early in vitro experiments demonstrated that amylin could inhibit glycogen synthesis and insulin-stimulated glucose uptake in isolated rat soleus muscle strips [4,5]. Amylin was thus proposed as a counter-regulatory hormone to insulin, antagonising insulin's action in peripheral rat tissues [6]. In rat soleus muscle amylin raises cAMP levels and thereby effects conversion of phosphorylase b to phosphorylase a and of glycogen synthase a to glycogen synthase b thus accounting for its effect to inhibit glycogen synthesis and promote glycogenolysis. [7–9]. These effects in turn explain amylin's induction of hyperlactataemia and hyperglycaemia in rats [10,11]. Amylin has been shown to cause insulin resistance in rats in vivo, although at concentrations greater than those found physiologically or in pathological situations [13–17]. However, the selective amylin antagonist AC187 markedly decreases the hyperlactataemia which accompanies hyperglycaemia in rats, suggesting that amylin released in response to hyperglycaemia is hyperlactataemic [12]. In humans amylin does not modify glucose metabolism during an i.v. glucose tolerance test (GTT) [18], or during a euglycaemic clamp [19]. In contrast, in euglycaemic clamp studies in rats, intravenous (i.v.) infusions of amylin can inhibit glucose uptake and decrease the effect of insulin to suppress hepatic glucose output [15,16]. These effects have been ascribed to amylin's effects on the liver [15] or on skeletal muscle [13]. It is clear that despite the relative controversy surrounding the pathophysiol-

ogical role of amylin in insulin resistance in humans, various animal models can provide a means of assessment of its activity.

We have recently presented a new compound, S15261, for the treatment of insulin resistance syndrome [20]. In chronic studies in ageing insulin resistant Sprague–Dawley rats S15261 lowered plasma insulin, triglyceride and cholesterol levels and restored impaired glucose tolerance. Euglycaemic glucose clamp studies showed these effects to be related to an increase in peripheral glucose uptake without modification of hepatic glucose production [20]. We also demonstrated that when the compound is infused acutely into the portal vein at very low doses (20–70 nmol/kg/h) it increases the glucose disappearance rate during an i.v. GTT.

Since amylin is co-stored and co-secreted with insulin, and is thus secreted directly into the portal vein, we undertook the present study to examine whether amylin could induce a state of insulin resistance when injected directly into this vein, instead of being injected at a peripheral site, and to assess the effects of S15261 under these circumstances. We report here that acute administration of amylin can impair glucose tolerance and that this effect is antagonised by S15261.

2. Experimental

2.1. Materials

Ketamine hydrochloride (Imalgene) was purchased from Rhône-Merieux (Lyon, France) and rat amylin amide was from Bachem (Basel, Switzerland). Other reagents and biochemicals were from Sigma. S15261, the L-isomer of 3-[2-[2-[4-[2-[α -fluorenyl acetyl amino ethyl]-benzoyloxy]ethyl amino]1-methoxy ethyl] trifluoromethylbenzene [20], and S15402, the L-isomer of [benzoyloxy ethyl amino]1-methoxy ethyl] trifluoromethylbenzene, were synthesized in-house and are shown in Fig. 1.

2.2. Animals

Male Sprague–Dawley rats of 3 months of age were used in these studies. They were maintained at a constant temperature (21°C) with a 12-h artificial light cycle, and had free access to water and standard laboratory chow (AO3, UAR Laboratory chow, Epinay, Villemuisson, France). Fifteen days prior to experimentation animals were anaesthetised with ketamine and a 0.2 mm (internal diameter) silastic catheter was implanted into the portal vein. Three days prior to the experiment a cardiac catheter was implanted. Finally, animals were fasted for 18 h before the experiments. Body weight loss due to surgical procedures was in the region of 10–12 g in all groups. All experimental procedures were approved by the Comité d'Ethique de l'Institut de Recherche Servier, and were carried out in compliance with French law regulating animal experimentation (Decree No. 87-848 19th October 1987, and the Ministerial Decrees of 19 April 1988).

2.3. Portal infusion and IVGTT

The methods for the implantation of catheters, the portal infusion technique and the simultaneous IVGTT were as described previously [20,21]. Briefly, animals with implanted silastic catheters into the portal and jugular veins receive on day-0 a one-hour portal infusion (0.035 ml/min) with saline, and three days later a one-hour portal infusion (0.035 ml/min) of amylin in the presence or absence of S15261. During the one-hour infusions an IVGTT is performed 30 min after beginning

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the portal infusion by injection of glucose (500 mg/kg) and serial blood sampling for the following 30 min during which the portal infusion is maintained. The rate of glucose disappearance (K_G) during the IVGTT is the slope of the decrease calculated by means square deviations [20,21].

2.4. Metabolite and hormone determinations

Plasma glucose was measured in a COBAS Mira S analyser (Roche Diagnostic Systems, Neuilly, France). Insulin was measured by radioimmunoassay using a Phasedeph kit (Kabi Pharmacia Diagnostics, St. Quentin-en-Yvelines, France).

2.5. Statistics

Data are presented as means \pm S.E.M. and were analysed using a Student's *t*-test.

3. Results

In these experiments amylin was infused at doses of 2.6 and 26 nmol/kg/h which we chose on the basis of reported data [15]. Amylin infused at a rate of 2.6 nmol/kg/h was without effect on basal plasma glucose (not shown) or insulin levels (see the first 30 min period in Fig. 2). There were also no differences in peak glucose or insulin levels following the i.v. glucose load. In contrast the K_G values (Table 1) were decreased by amylin by 67% at the higher dose, but there was no effect at the lower dose. Thus, amylin impaired glucose tolerance in normal rats.

The insulin response to glucose seemed modified by the infusion of amylin at 26 nmol/kg/h (Fig. 2), but no effect was seen at the lower dose (not shown). Thus, whilst basal or peak insulin levels were not altered by amylin, the return to basal values was much slower. Insulin levels in NaCl controls had returned to basal by T_{60} – T_{70} , whereas in the amylin group, insulin levels were still much higher (by 40–50%) at these times and had not returned to basal even at T_{120} . Therefore, the impaired glucose tolerance produced by amylin and shown in Table 1, took place despite a seemingly compensatory increase in insulin secretion, thereby, demonstrating the extent to which amylin had caused a transient insulin resistant state.

Infusion of S15261 at a dose shown to reverse impaired glucose tolerance in ageing insulin resistant rats (70 nmol/kg/h, [20]) together with amylin (at the higher dose) normalised K_G values (Table 1) but did nothing to the changes in insulin secretion produced by amylin (Fig. 2). S15261 by itself was also without effect on insulin secretion. In contrast administration of S15402 had no effect on the K_G values (Table 1) or on the insulin secretory profile (not shown).

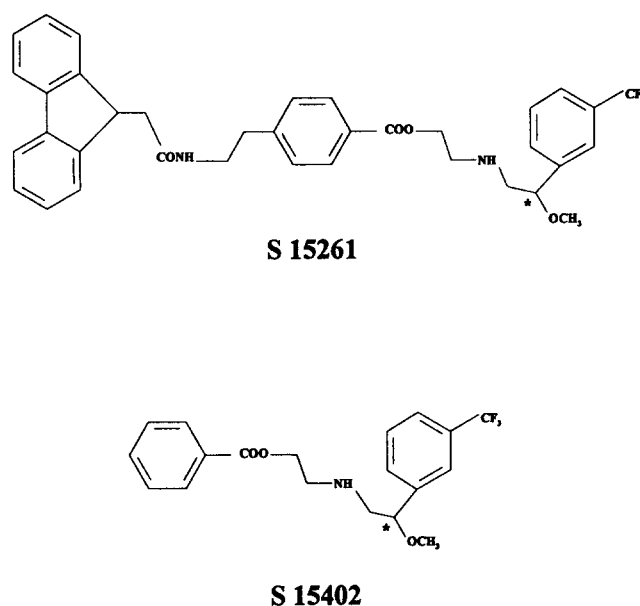


Fig. 1. Structures of S15261 and S15402. The asterisks indicate an asymmetric carbon.

4. Discussion

The intraportal infusion technique coupled to an IVGTT can be used to assess the acute effects of a compound on glucose tolerance. Whilst it is not as fully informative as a euglycaemic clamp, it is quicker to perform and provides an indication of a drug's effects on glucose metabolism. We have previously presented such studies with two types of drugs, benfluorex [21] and S15261 [20]. The data presented here demonstrate that an intraportal infusion of amylin can cause a transient state of insulin resistance in normal animals, and thus confirm previous observations using different methodology [13–16]. We have thus used the portal infusion method as a model of testing the effects of an insulin sensitising agent in an acute manner instead of in chronic studies.

The most important observation presented here is that S15261 antagonised the effects of amylin to impair glucose tolerance. The dose of S15261 chosen for this study is that shown to restore normal glucose tolerance in ageing insulin resistant Sprague–Dawley rats [20]. In previous euglycaemic clamp studies we showed the compound acts to increase peripheral glucose uptake, without effects on hepatic glucose output [21]. The data presented here support this notion as S15261 normalised K_G values despite having no effect on amylin's effects on the insulin secretory response to glucose (Fig. 2). Moreover, S15261 had no effects on insulin secretion at basal level either. These observations suggests that the effects of the drug are primarily at peripheral sites, probably at the level of skeletal muscle.

One could further suggest that, in the light of the antagonism of its effects by S15261, the action of amylin might also be at the level of skeletal muscle. Controversy exists over the effects of amylin on hepatic glucose metabolism. Thus, whilst Koopmans et al. [15] proposed that liver was the predominant organ of regulation by amylin, Frontoni and co-workers [13] suggested that muscle accounted for all of amylin's effects to

Table 1
Effects of an intraportal infusion of amylin, in the presence or absence of S15261 and S15402 on glucose disappearance rates during an IVGTT

Amylin (nmol/kg/h)	NaCl	S15261 (70 nmol/kg/h)	S15402 (70 nmol/kg/h)
None	3.54 \pm 0.24	3.45 \pm 0.50	3.30 \pm 0.45
2.6	3.42 \pm 0.40	—	—
26	1.71 \pm 0.27**	3.80 \pm 0.38**	2.27 \pm 0.13

Glucose disappearance rates are given as the K_G values ($\times 10^{-2}$) \pm S.E.M. for 6–8 animals. Statistical significance is given by ** $P < 0.01$ for the effects of amylin and by *** $P < 0.01$ for the effects of the drug versus the saline group.

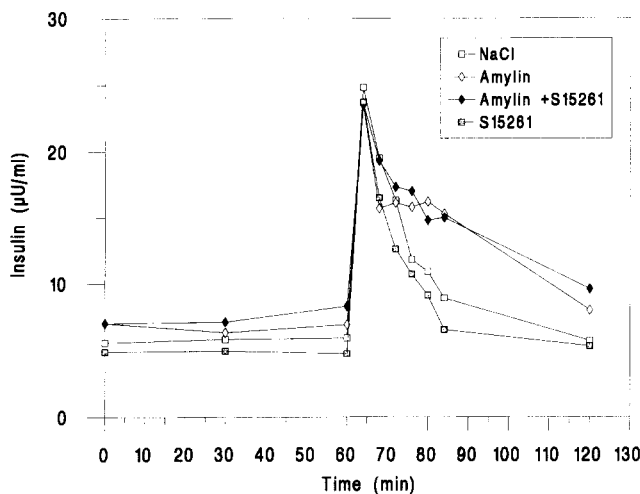


Fig. 2. Effects of an intraportal infusion of amylin in the absence or presence of S15261. Amylin was infused at 26 nmol/kg/h, and S15261 at 70 nmol/kg/h. The arrow indicates the time of injection of an i.v. glucose load, as per section 2. Error bars have been omitted for clarity, but in no case were they greater than 11%. Basal insulin levels were 6 ± 1 μ U/ml for NaCl controls, 7 ± 1 μ U/ml for the Amylin group, 5 ± 1 μ U/ml for the S15261 group and 7 ± 1 μ U/ml for the Amylin + S15261 group. The corresponding T_{120} values were 6 ± 1 , 8 ± 1 , 5 ± 1 , and 10 ± 1 μ U/ml.

induce insulin resistance in vivo. In vitro experiments using rat hepatocytes have shown either an absence of effects by amylin on insulin-stimulated glucokinase gene expression [22] or a direct effect of amylin to stimulate glycogenolysis and gluconeogenesis [23]. Furthermore, despite the presence of amylin receptors in liver membranes and in a population of hepatic cells [24–26], Stephens et al. [26] failed to demonstrate a direct effect of amylin on hepatic glucose metabolism. Finally, Nishimura and co-workers reported a lack of effect of amylin on hepatic glucose output in a perfused liver preparation [27].

Amylin has been reported to decrease the insulin secretory response to hyperglycaemia possibly by a paracrine effect [28]. In the present studies amylin did not inhibit glucose-induced insulin secretion, but on the contrary appeared to stimulate it at the higher dose used. It is possible that a portal infusion of amylin produces a pancreatic response more similar to that seen in perfused pancreas preparations where amylin causes either no effect [29] or a stimulation [30] of insulin secretion.

We cannot at present propose a mechanism of action for the effects of S15261 on amylin-induced impaired glucose tolerance. The data obtained with S15402, a compound of the same series as S15261 but with a reduced activity in animal models of insulin resistance (unpublished observations), supports the idea of a specific effect of S15261. In the light of its profile of activity we would argue that the effects of the drug are primarily at the level of glucose metabolism in skeletal muscle. Since amylin's effects on glucose metabolism also involve direct effects on skeletal muscle it may be tempting to speculate as to whether the activity of the drug may, in part, be related to an antagonism of the effects of amylin in this tissue. Experiments in isolated rat soleus muscle are underway to attempt to answer this question.

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