Investigation of the mechanism(s) of 8-OH-DPAT-mediated inhibition of plasma insulin in spontaneously hypertensive rats

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- 1 Effects of the prototype selective 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-n-dipropylamino)tetralin (8-OH-DPAT), were studied on the glycaemia and insulinaemia in conscious spontaneously hypertensive (SH) rats concurrently with blood pressure (BP) and heart rate (HR); underlying mechanism(s) were investigated in anaesthetized and pithed SH rats and in the perfused rat pancreas.
- 2 Intravenous (i.v.) injections of 8-OH-DPAT (150 µg kg⁻¹, i.v.) into fasted conscious but not anaesthetized SH rats increased glycaemia; glucose-stimulated (i.v. glucose tolerance test) plasma insulin levels were significantly inhibited in both cases without significant changes in glucose tolerance. Metabolic changes were associated with prominent decreases in BP and HR.
- 3 No inhibitory effect of 8-OH-DPAT, 150 µg kg⁻¹ i.v., on glucose-stimulated plasma insulin was observed in pithed SH rats; in contrast, clonidine (8 µg kg⁻¹ i.v.), produced marked inhibition of insulin levels in association with glucose intolerance. Neither compound decreased BP; rather, pronounced vasopressor effects were observed.
- 4 In the isolated perfused pancreas of the rat, 8-OH-DPAT, at 10⁻⁸ and 10⁻⁷ M, concentrations known to activate 5-HT_{1A} receptors *in vitro*, failed to modify glucose-stimulated insulin release. Inhibition (39 ± 7%) was seen only at a high concentration of 10⁻⁶ M.
- 5 The present data suggest that like the cardiovascular effects of 8-OH-DPAT, the inhibition of glucose-stimulated insulin release is mediated via the central nervous system. However, it is suggested that different mechanisms are involved in the cardiovascular actions and metabolic effects of 8-OH-DPAT in the SH rat; the latter are likely to reflect a consequence of activation of the hypothalamic-adrenal axis.

Introduction

It is now clearly established that the 5-hydroxytryptamine (5-HT) receptor family is very heterogeneous (for reviews see Fozard 1987; Peroutka, 1988). Three major classes of 5-HT receptors were identified by Bradley *et al.* (1986) and were designated 5-HT₁-like, 5-HT₂ and 5-HT₃; the 5-HT₁-like category, is now recognised to consist of at least four subtypes designated 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D}. Discriminatory ligands, tissue distributions and coupling to transduction mechanisms of the various 5-HT receptor subtypes have now been well-documented (Peroutka, 1988; Hoyer, 1989).

The availability of compounds with high affinities and certain selectivities has greatly increased our understanding of the functional significance of some of the above 5-HT receptor subtypes (Hibert *et al.*, 1990). In particular, an impressive number of 5-HT_{1A} receptor-mediated functional/biological responses have been established (Dourish *et al.*, 1987; Hibert *et al.*, 1990); this remarkable progress has been possible, primarily, due to the discovery that the centrally acting 5-HT receptor agonist, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) (Hjorth *et al.*, 1982), has high affinity and selectivity for the 5-HT_{1A} subtype of the 5-HT₁ recognition site (Middlemiss & Fozard, 1983; Gozlan *et al.*, 1983). One of the several functional responses elicited by 8-OH-DPAT is to lower blood pressure and heart rate in a number of species. The cardiovascular effects of 8-OH-DPAT have been well characterized and are mediated via activation of postsynaptic 5-HT_{1A} receptors in the central nervous system resulting in an increase in vagal tone and sympathoinhibition; in the rat, moreover, there is evidence for an indirect involvement of α₂-adrenoceptors (for review see Mir & Fozard, 1987; 1990).

More recently, it has become evident that central 5-HT_{1A} receptor activation also results in stimulation of the pituitary-adrenal axis. Thus, for example, 8-OH-DPAT has been shown

to increase plasma prolactin, ACTH, corticosterone and β-endorphin levels and to decrease growth hormone levels in rats (Simonovic *et al.*, 1984; Koenig *et al.*, 1987; Gilbert *et al.*, 1988; Aulakh *et al.*, 1988). The effects of 8-OH-DPAT on endocrine/metabolic parameters are further reflected in its ability to promote hyperglycaemia and to inhibit insulinaemia in response to an intravenous glucose tolerance test in conscious Sprague-Dawley rats (Chaouloff & Jeanrenaud, 1987); on the basis of pharmacological analysis these effects were considered to be mediated via 5-HT_{1A} and α₂-adrenoceptors. Further evidence for marked inhibitory effects of 8-OH-DPAT on both the basal hyperinsulinaemia and glucose-stimulated insulin release was obtained in the conscious genetically obese (fa/fa) rat (Chaouloff & Jeanrenaud, 1988).

Despite certain similarities concerning the receptor sites involved, the hyperglycaemic and hypoinsulinaemic effects of 8-OH-DPAT are not entirely consistent with its mode of action on the autonomic nervous system i.e. sympathoinhibition and increase in vagal tone. It is generally recognised, for instance, that a physiological consequence of both an increase in vagal tone and sympathoinhibition in several species is an increase in insulinaemia associated with a decrease in glycaemia (Woods & Porte, 1974; Ahren *et al.*, 1986). It is likely, therefore, that different mechanisms are involved in the manifestation of the above endocrine/metabolic effects and the cardiovascular actions of 8-OH-DPAT. The objectives of the present study were to investigate the effects of 8-OH-DPAT on basal and glucose-stimulated (i.v. glucose tolerance test) plasma glucose and insulin levels concurrently with monitoring of blood pressure and heart rate in conscious spontaneously hypertensive (SH) rats. In addition, experiments were conducted in anaesthetized and pithed SH rats as well as isolated perfused pancreas of the rat to elucidate the mechanism(s) underlying the hypoinsulinaemic effect of 8-OH-DPAT. Some of these results was presented to the British Pharmacological Society in July, 1989 (Bouhelal *et al.*, 1989).

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Methods

Animals

Male SH rats were supplied by Tier Farm Madörin, Füllinsdorf, Switzerland, and male Wistar rats by Charles River France. All animals had free access to food and water and were maintained on a constant 12 h light cycle.

Glucose tolerance tests in conscious SH rats

Male spontaneously hypertensive (SH) rats (300–350 g) were used for these experiments. Catheters were implanted under sodium-hexobarbitone (NA-Evipan) anaesthesia (160 mg kg⁻¹, i.p.) in the abdominal aorta and femoral vein. Cannulae were passed subcutaneously and exteriorized at the back of the neck. At the end of surgery, cannulae were filled with heparinized saline to avoid blood clotting. Experiments were performed about one week after surgery.

Following an overnight fast (18–20 h) blood pressure (BP) and heart rate (HR) were recorded continuously from the arterial cannula as described in detail previously (Fozard *et al.*, 1987). After a stabilization period of about 1 h an arterial blood sample (200 µl) was taken to establish basal glucose and insulin values. Subsequently saline (1 ml kg⁻¹, i.v.) or 8-OH-DPAT (150 µg kg⁻¹, i.v.) was injected via the venous cannula and another blood sample taken 15 min later to determine the acute effects of saline or 8-OH-DPAT on resting glucose and insulin concentrations. Glucose (0.5 g kg⁻¹ in 0.5 ml water) was then injected i.v. and further blood samples withdrawn after 1, 6, 12, 24, and 48 min.

Time-dependent effects of 8-OH-DPAT *per se* on basal glycaemia, insulinaemia, BP and HR were determined in a separate series of experiments. A blood sample was taken at time 0 to determine basal glucose and insulin values, subsequently saline (1 ml kg⁻¹, i.v.) or 8-OH-DPAT (150 µg kg⁻¹, i.v.) was injected and further blood samples withdrawn after 5, 15, 30 and 60 min. The arterial cannula was rinsed with heparinized saline following each blood sampling and care was taken not to dilute the sample with the residual saline in the cannula by discarding the first few drops.

Glucose tolerance tests in anaesthetized SH rats

Male SH rats fasted overnight (18–20 h) were anaesthetized with 120 mg kg⁻¹ i.p. of sodium hexobarbitone (Inactin) and cannulae placed in a jugular vein and a carotid artery. An arterial blood sample (200 µl) was taken to establish basal glucose and insulin values. Subsequently saline (1 ml kg⁻¹, i.v.) or 8-OH-DPAT (150 µg kg⁻¹, i.v.) was injected and further samples withdrawn 5, 10 and 15 min later to determine the acute effects of saline or 8-OH-DPAT on resting glucose and insulin values. Glucose tolerance tests were then performed as described above for conscious SH rats. In a separate series of experiments time-dependent effects of 8-OH-DPAT (150 µg kg⁻¹, i.v.) or saline (1 ml kg⁻¹, i.v.) on basal glycaemia and insulinaemia were determined by withdrawing blood samples before injection (0 time) and 5, 10, 16, 21, 27, 39, and 63 min after injection.

Glucose tolerance tests in pithed SH rats

Non-fasted SH rats (300–350 g) were anaesthetized with sodium pentobarbitone (40 mg kg⁻¹, i.p. supplemented by s.c. injection) and pithed according to the procedure described previously (Fozard *et al.*, 1987) based on the method of Shipley & Tilden (1947). A tracheotomy was performed and rats were artificially ventilated with pure oxygen. Catheters were inserted in the right femoral vein and artery. Experiments were generally initiated between 35–45 min after pithing, to allow the preparations to stabilize. Effects of 8-OH-DPAT (150 µg kg⁻¹, i.v.) and clonidine (8 µg kg⁻¹, i.v.)

on glucose tolerance were determined as described for conscious and anaesthetized animals.

Glucose-induced insulin secretion from perfused rat pancreas in vitro

Pancreases from male Wistar rats (320–380 g) anaesthetized with sodium pentobarbitone 60 mg kg⁻¹ (i.p.), were isolated and perfused as described previously (Loubatieres *et al.*, 1969). Each pancreas was transferred to a plastic perfusion chamber maintained at 37.5°C, and perfused through its own arterial system with a Krebs Ringer bicarbonate solution containing (mm): NaCl 108, KH₂PO₄ 1.19, KCl 4.74, CaCl₂ 2.74, MgSO₄ • 7H₂O 1.19, bovine serum albumin 2 g l⁻¹ (Fraction V, Sigma Chemical Company) and glucose 1.5 g l⁻¹ (8.3 mM). This glucose concentration was chosen to induce a moderate insulin secretion. The perfusion fluid was continuously bubbled with a mixture of CO₂ (5%) and O₂ (95%) in order to maintain pH around 7.35. The perfusion pressure was chosen so as to produce an output flow rate of 2.5 ml min⁻¹ at the time of addition of drugs (45 min). The first sample was taken after an equilibration period of 30 min. Two additional samples were taken 10 and 15 min later in the presence of glucose (8.3 mM) alone. 8-OH-DPAT at various concentrations (10⁻⁸ M, 10⁻⁷ M or 10⁻⁶ M) was then added to the perfusion fluid and samples were collected every minute for 5 min then 8, 10, 15, 20 and 30 min after starting 8-OH-DPAT administration. Samples were frozen at -20°C until insulin was determined. Insulin output was obtained by multiplying the hormone concentration in the effluent (µm l⁻¹) by the flow rate (ml min⁻¹).

Plasma glucose and insulin measurements

Blood samples were collected in Eppendorf tubes through the arterial cannula and kept at 4°C before centrifugation (8,800 *g* for 3 min). Aliquots (10 µl) of the plasma were used to determine glucose concentrations in an automatic glucose analyser (Kodak, Ektachem). The remainder of the plasma was stored at -20°C for subsequent insulin assay. Insulin was determined by radioimmunoassay as described by Hales & Randle (1963).

Drugs and solutions

(±)-8-OH-DPAT hydrogen bromide and clonidine hydrochloride (Research Biochemicals Inc.) were dissolved in 0.9% (w/v) NaCl for *in vivo* experiments. Human [¹²⁵I]-insulin (Amersham International) and anti-insulin antiserum raised in guinea-pigs was from Medipro (Basel, Switzerland).

Statistical analysis

Statistical analysis of data was performed by analysis of variance followed by Mann Whitney U test for unpaired data and differences were considered significant at the *P* < 0.05 level.

Results

Effects of 8-OH-DPAT on basal and glucose-induced plasma glucose and insulin levels, blood pressure and heart rate in conscious SH rats

The dose of 150 µg kg⁻¹ i.v. has been previously characterized in detail for the hyperglycaemic and hypoinsulinaemic effects of 8-OH-DPAT in rats (Chaouloff & Jeanrenaud, 1987; 1988); therefore, this dose was used in the present study. Administration of 8-OH-DPAT, 150 µg kg⁻¹ i.v., resulted in an increase in basal glycaemia (32 ± 6 mg dl⁻¹) which was maximal at 15 min, and was back to control values by 60 min

Figure 1 Effects of 8-OH-DPAT on basal plasma glucose and insulin levels in fasted conscious SH rats. Blood samples were withdrawn to estimate basal plasma glucose and insulin levels before saline or 8-OH-DPAT ($150 \mu\text{g kg}^{-1}$, i.v.) administration. Further samples were taken at the times indicated in the figure. Open column: control group ($n = 6$), hatched columns: 8-OH-DPAT treated group ($n = 6$). Results are expressed as mean with s.e.mean shown by vertical bars. * $P < 0.05$, value significantly different from the corresponding control value.

Figure 2 Effects of 8-OH-DPAT on plasma glucose and insulin in fasted conscious SH rats following a glucose load. Plasma glucose and insulin values were determined before 8-OH-DPAT ($150 \mu\text{g kg}^{-1}$, i.v.) or saline injections. Acute effects of the drug were determined 15 min post injection. Glucose (0.5 g kg^{-1} , i.v.) was then injected at time 0 and subsequent blood samples were withdrawn for glucose and insulin determinations at the times indicated. Open columns: control group ($n = 5$), hatched columns: 8-OH-DPAT-treated group ($n = 8$). Results are represented as mean with s.e.mean shown by vertical bars. * $P < 0.05$, value significantly different from the corresponding control value. For further details of the experimental protocol, see text.

Figure 3 Effects of 8-OH-DPAT on blood pressure and heart rate during glucose tolerance tests in fasted conscious SH rats. Rats were injected with either saline or 8-OH-DPAT $150 \mu\text{g kg}^{-1}$, i.v. and 15 min later subjected to an intravenous glucose tolerance test as described in the text. Changes in mean BP and HR are represented as mean with s.e.mean shown by vertical bars. (●): Control group ($n = 5$), (■): 8-OH-DPAT-treated group ($n = 8$). Resting BP and HR values (mean $\pm$ s.e.mean) respectively for control: 185 ± 10 ; 340 ± 13 and 8-OH-DPAT-treated group: 193 ± 5 ; 362 ± 10 .

(Figure 1). The increase in glycaemia was associated with a small but variable increase in basal insulinaemia (Figure 1).

In a separate series of experiments the effect of 8-OH-DPAT on an intravenous glucose tolerance test was determined. 8-OH-DPAT ($150 \mu\text{g kg}^{-1}$ i.v.) increased the basal glycaemia, $100 \pm 6 \text{ mg dl}^{-1}$, to $146 \pm 13 \text{ mg dl}^{-1}$ 15 min post injection with no effect on the basal insulinaemia (Figure 2). Following the intravenous injection of glucose (0.5 g kg^{-1}), plasma glucose levels in the saline-treated animals increased to $281 \pm 9 \text{ mg dl}^{-1}$ and returned to control levels within about 24 min. The increase in glycaemia was associated with a rapid increase in plasma insulin from 28 ± 5 to $326 \pm 58 \mu\text{u ml}^{-1}$ (Figure 2). In contrast, the 8-OH-DPAT treated group showed a marked inhibition ($49 \pm 9\%$) in the glucose-stimulated peak insulin levels; this inhibition was not accompanied by any further increase in the glycaemia above the control levels if the increase in glycaemia caused by 8-OH-DPAT *per se* is taken into account (cf. Figures 1 and 2). Injection of 8-OH-DPAT in these animals resulted in marked and sustained decreases in BP and heart rate whereas injections of saline (1 ml kg^{-1} , i.v.) or glucose (0.5 g kg^{-1} , i.v.) had no significant effects on either parameter (Figure 3).

Effects of 8-OH-DPAT on basal and glucose-induced plasma glucose and insulin levels in anaesthetized SH rats

In contrast to conscious SH rats, in anaesthetized SH rats 8-OH-DPAT ($150 \mu\text{g kg}^{-1}$, i.v.) had little effect on the basal plasma glucose levels; the basal levels of insulin also showed no significant change (Figure 4). The resting levels of glucose and insulin in the anaesthetized animals were similar to the levels in conscious animals.

The effects of 8-OH-DPAT on glucose-stimulated plasma insulin and glucose were determined in a separate series of experiments. Again, 8-OH-DPAT ($150 \mu\text{g kg}^{-1}$ i.v.) had no effect on basal plasma glucose or insulin levels measured

Figure 4 Effects of 8-OH-DPAT on basal plasma glucose and insulin levels in fasted anaesthetized SH rats. Blood samples were withdrawn to determine basal plasma glucose and insulin levels prior to injection of saline or 8-OH-DPAT ($150 \mu\text{g kg}^{-1}$, i.v.). Further samples were taken at the times indicated in the figure. Open columns: control group ($n = 3$), hatched columns: 8-OH-DPAT-treated group ($n = 4$). Results are expressed as mean with s.e.mean shown by vertical bars. For further details of the experimental protocol, see text.

15 min post injection (Figure 5). Following a glucose load (0.5 g kg^{-1} , i.v.), plasma glucose and insulin levels in the saline treated animals increased rapidly within 1 min to $407 \pm 18 \text{ mg dl}^{-1}$ and $231 \pm \mu\text{u ml}^{-1}$ respectively and returned to control values rather slowly as compared to the conscious animals (cf. Figures 2 and 5). 8-OH-DPAT-treated rats showed a significant reduction in the plasma insulin levels at 1 and 6 min compared to the saline-treated animals whereas

Figure 5 Effects of 8-OH-DPAT on plasma glucose and insulin in fasted anaesthetized SH rats following a glucose load. Basal plasma glucose and insulin values were determined prior to injection of saline or 8-OH-DPAT ($150 \mu\text{g kg}^{-1}$, i.v.). Acute effects of the drug were determined 15 min post injection. Glucose (0.5 g kg^{-1} , i.v.) was then injected at time 0 and subsequent blood samples withdrawn at the times indicated. Open columns: control group ($n = 5$), hatched columns: 8-OH-DPAT-treated group ($n = 6$). Results are expressed as mean with s.e.mean shown by vertical bars. * $P < 0.05$, value significantly different from the corresponding control value.

Figure 6 Effects of 8-OH-DPAT on plasma glucose and insulin levels in pithed SH rats following a glucose load. Basal plasma glucose and insulin values were determined 35 to 45 min after pithing prior to saline or 8-OH-DPAT ($150 \mu\text{g kg}^{-1}$, i.v.) injections. Acute effects of 8-OH-DPAT were determined 15 min post injection. Glucose (0.5 g kg^{-1} , i.v.) was then injected at time 0 and subsequent blood samples withdrawn at the time indicated. Open columns: control group ($n = 6$), hatched columns: 8-OH-DPAT-treated group ($n = 4$). Results are expressed as means with s.e.mean shown by vertical bar. * $P < 0.05$, value significantly different from the corresponding control value.

the glucose levels were almost identical (Figure 5). A reduction in blood pressure (resting value: $163 \pm 12 \text{ mmHg}$; after injection of 8-OH-DPAT: $70 \pm 7 \text{ mmHg}$) and in heart rate (resting value: 324 ± 16 ; 8-OH-DPAT: $254 \pm 14 \text{ beats min}^{-1}$) was also observed with 8-OH-DPAT in the anaesthetized SH rats. However, a significant decrease in blood pressure (resting: $157 \pm 18 \text{ mmHg}$; 12 min after saline injection: $100 \pm 12 \text{ mmHg}$) but not heart rate (resting: $325 \pm 9 \text{ beats min}^{-1}$; saline $306 \pm 21 \text{ beats min}^{-1}$) was also observed in the saline-treated rats which appeared to be associated with Inactin anaesthesia.

Effects of 8-OH-DPAT and clonidine on glucose-induced plasma glucose and insulin levels in pithed SH rats

For these experiments, experimental conditions were modified as compared to the Inactin-anaesthetized SH rat model. First, Inactin was replaced by sodium pentobarbitone as the anaesthetic. Second, non-fasted animals were used instead of fasted animals. The reasons for these changes were that Inactin anaesthesia and fasting prior to pithing caused marked decreases in blood pressure ($< 30\text{--}40 \text{ mmHg}$) and basal glycaemia ($40\text{--}80 \text{ mg dl}^{-1}$), thus making the preparations unstable. However, it was found that under the conditions where the animals were anaesthetized with pentobarbitone and not fasted, the pithed preparations were stable throughout the duration of the experiment. Under these conditions resting blood pressure and glucose levels were respectively $63 \pm 5 \text{ mmHg}$ ($n = 6$) and $148 \pm 15 \text{ mg dl}^{-1}$ ($n = 6$); the basal plasma insulin in pithed animals was significantly increased compared to fasted Inactin-anaesthetized animals. Similar

Table 1 Effect of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) and clonidine on blood pressure (mmHg) in pithed SH rats

	Baseline	Maximal effect	n
Control	63 ± 5	72 ± 8	6
8-OH-DPAT	69 ± 6	99 ± 12(*)	4
Clonidine	63 ± 5	166 ± 12(*)	5

Effects of saline, 8-OH-DPAT ($150 \mu\text{g kg}^{-1}$, i.v.) and clonidine ($8 \mu\text{g kg}^{-1}$, i.v.) on mean blood pressure in pithed SH rats. Data were obtained from the same experiments shown in Figures 6 and 7 and are expressed as absolute values, mean $\pm$ s.e. mean of the number of animals indicated in the table (n). * $P < 0.05$; value significantly different from the corresponding baseline value.

glucose concentrations were found in sodium pentobarbitone-anaesthetized and conscious SH rats in the non-fasting state (results not shown).

8-OH-DPAT ($150 \mu\text{g kg}^{-1}$, i.v.) had no significant effects on basal glucose levels or the glycaemia induced by an intravenous glucose tolerance test (Figure 6). In contrast to conscious and anaesthetized animals, no inhibition of glucose-stimulated plasma insulin levels was observed in pithed animals after 8-OH-DPAT administration; if anything a significantly higher level of plasma insulin was observed at 6 min after a glucose load in 8-OH-DPAT treated animals. 8-OH-DPAT elicited vasoconstrictor activity in pithed SH rats which lasted 7 ± 2 min and no decreases in blood pressure were observed (Table 1).

In a separate series of experiments the effects of clonidine on basal and glucose-stimulated plasma glucose and insulin concentrations were investigated. As shown in Figure 7, cloni-

Figure 7 Effects of clonidine ($8 \mu\text{g kg}^{-1}$, i.v.) on plasma glucose and insulin levels in pithed SH rats following a glucose load. The experimental protocol is the same as described in the legend to Figure 6. Open columns: control group (n = 6), hatched columns: clonidine-treated group (n = 5). Results are expressed as means with s.e. mean shown by vertical bars. * $P < 0.05$, value significantly different from the corresponding control value. **Figure 8** Effect of 8-OH-DPAT on insulin secretion from isolated perfused pancreas of the rat. The perfusion medium contained 8.3 mM (1.5 g l^{-1}) of glucose. Results are expressed in percentages in relation to the value at time 45 min taken as reference. Values are mean of 4 to 6 experiments with s.e. mean shown by vertical bars. At time 45 min, mean insulin output rates were 709 ± 134 (n = 6), 551 ± 98 (n = 6), 756 ± 76 (n = 6) and 673 ± 71 (n = 4) $\mu\text{u min}^{-1}$ respectively for control (■) and 8-OH-DPAT-treated tissues (●), 10^{-8} M (a), 10^{-7} M (b) and 10^{-6} M (c).

dine ($8 \mu\text{g kg}^{-1}$, i.v.) caused a significant reduction in basal insulinaemia (from 101 ± 14 to $26 \pm 8 \mu\text{u ml}^{-1}$); this inhibition was associated with a significant increase in basal glycaemia after 15 min (from 138 ± 5 to $178 \pm 13 \text{ mg dl}^{-1}$). The decrease in insulin concentrations was also observed during the intravenous glucose tolerance test. Clonidine inhibited glucose-stimulated insulin levels by $88 \pm 4\%$ and caused impairment of the glucose tolerance (Figure 7). Like 8-OH-DPAT, clonidine also elicited vasoconstrictor activity in pithed SH rats lasting 28 ± 3 min and no decreases in blood pressure were observed (Table 1).

Effects of 8-OH-DPAT on glucose-induced insulin secretion from the isolated perfused pancreas

8-OH-DPAT, at two concentrations (10^{-8} and 10^{-7} M) did not modify glucose-induced insulin secretion from the isolated perfused pancreas (Figure 8); inhibition ($39 \pm 7\%$, of basal) was seen only at 10^{-6} M 8-OH-DPAT (Figure 8).

Discussion

The present results provide strong evidence that the 8-OH-DPAT-induced inhibition of glucose-stimulated plasma insulin in the SH rat is mediated via the central nervous system. This conclusion is based on two key observations. Firstly in the pithed SH rat, 8-OH-DPAT ($150 \mu\text{g kg}^{-1}$, i.v.)

did not elicit any inhibition of the glucose-stimulated insulinemia, rather a small augmentation was observed. In contrast both in the conscious and anaesthetized SH rat, 8-OH-DPAT (150 $\mu\text{g kg}^{-1}$, i.v.) inhibited the glucose-stimulated (i.v. glucose tolerance test) plasma insulin levels, suggesting a central mechanism of action. These effects were associated with profound decreases in blood pressure and heart rate. That the pithed rat preparation can detect agents inhibiting insulin release through peripheral mechanisms, i.e. at the level of the pancreas, is clear from the data obtained with clonidine which produced a marked and sustained inhibition of both basal and glucose-stimulated insulin levels which was associated with glucose intolerance. The hypoinsulinaemic effect of clonidine has been extensively studied *in vivo* (Angel *et al.*, 1988; Angel & Langer, 1988) and *in vitro* (Nakaki *et al.*, 1981; Hillaire-Buys *et al.*, 1985) and has been attributed to the activation of α_2 -adrenoceptors present on the pancreatic β -cells. The inability of 8-OH-DPAT to inhibit insulin levels in this preparation cannot be attributed to the difference in cardiovascular actions *vis à vis* conscious and anaesthetized rats, since clonidine, like 8-OH-DPAT, showed no blood pressure lowering activity in the pithed SH rat but inhibited insulin levels.

Secondly, indirect support for the action of 8-OH-DPAT being centrally mediated comes from the inability of 8-OH-DPAT at concentrations of 10^{-8} and 10^{-7} M to inhibit glucose-stimulated insulin release from the isolated perfused pancreas of the rat. Although some inhibition of insulin release was observed at 10^{-6} M 8-OH-DPAT, this is unlikely to be due to an interaction with 5-HT_{1A} receptors. Thus, *in vitro*, functional as well as biochemical responses to 8-OH-DPAT mediated via 5-HT_{1A} receptors, such as inhibition of contraction of the transmurally stimulated guinea-pig ileum (Mir *et al.*, 1988) and inhibition of adenylate cyclase (Bockaert *et al.*, 1987) are observed in the 10^{-8} – 10^{-7} M concentration range. In addition, methiotepin, a non-selective 5-HT_{1A} receptor blocker, at 10^{-7} M failed to antagonize the 8-OH-DPAT-mediated inhibition of glucose-stimulated insulin release from the isolated perfused pancreas (unpublished observation). 8-OH-DPAT has a certain affinity for α_2 -adrenoceptors (pIC_{50} 6.91; Fozard *et al.*, 1987) and it is theoretically possible that at high concentrations 8-OH-DPAT can inhibit insulin release via a mechanism similar to that of clonidine (see above) at the level of pancreatic β -cells. However, 8-OH-DPAT does not exhibit α_2 -adrenoceptor agonist activity in the transmurally stimulated guinea-pig ileum (Mir & Fozard, 1987) and seems to be an antagonist at these sites (Crist & Surprenant, 1987).

8-OH-DPAT did not induce hyperglycaemia in the presence of anaesthesia whereas the inhibition of evoked plasma insulin was unaffected. It appears, therefore, that anaesthesia selectively alters the mechanism(s) eliciting basal glycaemia in response to 8-OH-DPAT. An important feature of the metabolic effects of 8-OH-DPAT was that despite marked inhibition of glucose-stimulated insulin release, no worsening of the glucose tolerance was observed in either conscious or anaesthetized SH rats. In the conscious SH rat the net glycaemia induced by 8-OH-DPAT in the absence of glucose injection was similar to the difference in glycaemia between the control and 8-OH-DPAT-treated groups following the glucose injection (see for comparison Figures 1 and 2). These data suggest that despite inhibition of insulinemia enough of the hormone is present in the circulation for adequate tissue uptake of glucose. An alternative explanation could be that 8-OH-DPAT enhances insulin sensitivity and/or metabolism of glucose via a decrease in glucagon secretion. It is interesting that fenfluramine, a 5-HT releaser, improves insulin action in

patients with non-insulin dependent diabetes mellitus (NIDDM) (Pestell *et al.*, 1989), which is characterized by hyperinsulinaemia. The present data extend the results of Chaouloff & Jeanrenaud in conscious normoinsulinaemic (Chaouloff & Jeanrenaud, 1987) and genetically obese (fa/fa) rats (Chaouloff & Jeanrenaud, 1988), showing that 8-OH-DPAT increases basal glycaemia and inhibits glucose-stimulated insulin release without any adverse effects on glucose tolerance in either case. The data also support a central mechanism of action for 8-OH-DPAT. However, despite the metabolic and cardiovascular effects of 8-OH-DPAT being centrally mediated and involving pharmacologically similar receptor sites, 5-HT_{1A} and α_2 -adrenoceptors (Chaouloff & Jeanrenaud, 1987; Fozard *et al.*, 1987) it is unlikely that the mechanisms of action of 8-OH-DPAT on the cardiovascular system (increase in cholinergic tone and sympathoinhibition) can account for the hypoinsulinaemic and hyperglycaemic effects of 8-OH-DPAT; an increase in vagal tone and sympathoinhibition is known generally to favour the opposite response i.e. hyperinsulinaemia and hypoglycaemia (Woods & Porte, 1974; Ahren *et al.*, 1986).

Thus, in the context of the above discrepancy it is worthwhile considering recent evidence that central 5-HT_{1A} receptor activation also results in stimulation of the pituitary-adrenal axis manifested as changes in prolactin, ACTH, corticosterone, β -endorphin and growth hormone (see Introduction). The stimulation of the endocrine axis is likely to arise from the level of the hypothalamus, since 5-HT nerve terminals make synaptic connections in the paraventricular nucleus with corticotropin releasing factor (CRF)-containing neurones in the rat hypothalamus (Liposits *et al.*, 1987), and 5-HT is reported to enhance the release of CRF (Jones *et al.*, 1976). CRF, apart from producing adrenal stimulation of corticosteroids via the pituitary axis, also directly stimulates sympatho-adrenal tone, resulting in increased plasma adrenaline, and increases blood pressure (for review see Fisher, 1989). CRF also stimulates release of somatostatin (Peterfreund & Vale, 1983). Both somatostatin (Reichlin, 1986) and catecholamines are potent inhibitors of insulin release at the level of the pancreas and therefore 8-OH-DPAT-mediated inhibition of insulin release may involve actions of somatostatin and/or catecholamines as a consequence of CRF release. In this context, 8-OH-DPAT, 62–500 $\mu\text{g kg}^{-1}$ i.v., has been recently shown to increase plasma adrenaline and noradrenaline levels (presumed to be of adrenal origin) associated with transient increases in blood pressure which precede the decrease in blood pressure in conscious normotensive rats (Bagdy *et al.*, 1989).

In view of the foregoing it appears likely that the hypoinsulinaemic/hyperglycaemic effects of 8-OH-DPAT reflect activation of the hypothalamic-adrenal axis and may be mediated via action(s) of CRF, somatostatin, corticosterone and catecholamines. In support of this, in preliminary experiments in adrenalectomized, conscious normotensive rats, we have found that both the hyperglycaemic and hypoinsulinaemic effects of 8-OH-DPAT, as well as the initial pressor response, are abolished whereas the hypotensive effects were unaffected (Bouhelal and Mir, unpublished data). The effects of 8-OH-DPAT on cardiovascular parameters, glycaemia and insulinaemia are being evaluated in adrenalectomized rats to obtain further evidence for the concept that the metabolic and cardiovascular effects of 8-OH-DPAT are mediated via different mechanisms.

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