Role of the adrenal medulla in the metabolic and pressor actions of 8-OH-DPAT

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1 Intravenous administration of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT, 150 $\mu\text{g kg}^{-1}$) into conscious sham-operated rats caused significant increases in basal glycaemia with minor changes in basal insulinaemia. Glucose-stimulated (intravenous glucose tolerance test) plasma insulin levels were significantly inhibited in 8-OH-DPAT-treated sham-operated animals. These metabolic changes were associated with significant and sustained falls in blood pressure (BP) and heart rate (HR) preceded by transient (<5 min) increases only in BP.

2 In adrenodemedullated animals, 8-OH-DPAT failed to cause an initial vasoconstriction, hyperglycaemia, or inhibition of glucose-stimulated plasma insulin despite eliciting falls in BP and HR that were comparable to those observed in sham-operated animals.

3 Noradrenaline, adrenaline and dopamine levels in the adrenal tissue were reduced by about 95% in adrenodemedullated rats as compared to sham-operated rats. A functionally intact adrenal cortex was indicated by the presence of corticosterone in the plasma of both adrenodemedullated and sham-operated rats.

4 The present data demonstrate that 8-OH-DPAT mediates an initial increase in BP and changes in metabolic parameters via intact adrenal medulla and may thus be consequential to the release of adrenaline, whereas the sustained cardiovascular effects of 8-OH-DPAT are not.

Keywords: Adrenal medulla; catecholamines; insulin; glucose; 8-OH-DPAT; conscious Sprague-Dawley rats; blood pressure

Introduction

5-Hydroxytryptamine_{1A} (5-HT_{1A}) receptor agonists such as 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) and fleroxan decrease blood pressure (BP) and heart rate (HR) in many species through an interaction with central 5-HT_{1A}-receptors (Fozard *et al.*, 1987; Ramage & Fozard, 1987; Doods *et al.*, 1988; for review see Mir & Fozard, 1990). These actions are associated with an increase in vagal tone and sympathoinhibition (Ramage & Fozard, 1987; Ramage *et al.*, 1988).

8-OH-DPAT has been shown also to act on the endocrine system. For example, 8-OH-DPAT, through activation of 5-HT_{1A}-receptors, decreases plasma insulin and increases basal plasma glucose levels in several rat strains, via a central mechanism of action (Chaouloff & Jeanrenaud, 1987, 1988; Bouhelal *et al.*, 1990; Laude *et al.*, 1990).

5-HT_{1A}-receptor agonists enhance plasma levels of corticosterone (Koenig *et al.*, 1987; Aulakh *et al.*, 1988; Chaouloff *et al.*, 1990c; Bouhelal & Mir, 1990) and catecholamines (Badgy *et al.*, 1989; Chaouloff *et al.*, 1990a; Laude *et al.*, 1990). We have shown previously that despite the fact that the effects of 8-OH-DPAT on glucose and insulin are centrally mediated, they are dependent upon the adrenal glands being intact (Bouhelal & Mir, 1990). Similarly, the initial short-lasting pressor effect of 8-OH-DPAT (Gradin *et al.*, 1985; Fozard *et al.*, 1987; Bagdy *et al.*, 1989) is also dependent on intact adrenal glands (Bouhelal & Mir, 1990). Both parts of the adrenal gland (cortex and medulla) can participate in glucose metabolism, through their respective secretory products (corticosteroids and catecholamines). The objective of the present study was to investigate the importance of the medulla versus the cortex of the adrenal gland in the mediation of the metabolic (hyperglycaemia and inhibition of insulin release during a glucose tolerance test) and pressor effects of 8-OH-DPAT by using adrenodemedullated and sham-operated conscious Sprague-Dawley rats.

Methods

Animals

Adrenodemedullated and sham-operated Sprague-Dawley rats (300–380 g) were supplied by IFFA-CREDO (Les Oncins, France). They had free access to food and were given a solution of 1% saccharose and 0.9% NaCl to drink during the course of the study. Animals were maintained on a constant 12 h light cycle. Animals were used about 2 to 3 weeks after adrenodemedullation. Successful adrenodemedullation was assessed by measuring the adrenal catecholamines at the end of the experiments.

Intravenous glucose tolerance test (IVGTT) and cardiovascular recordings in conscious animals

Experiments were conducted with minor modification of the procedures described in detail previously (Bouhelal *et al.*, 1990). Briefly, catheters were implanted under sodium hexobarbitone (NA-Evipan) anaesthesia (160 mg kg⁻¹ i.p.) in the femoral artery and 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 at least three days after surgery. BP and 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 $\mu\text{g kg}^{-1}$ 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 plasma glucose and insulin concentrations. The dose of 150 $\mu\text{g kg}^{-1}$ i.v. of 8-OH-DPAT has been previously characterized in detail for the metabolic and cardiovascular effects in rats (Chaouloff & Jeanrenaud, 1987, 1988; Bouhelal *et al.*, 1990; Bouhelal & Mir, 1990); therefore, this dose was used in the present study. Glucose (0.5 g kg⁻¹ in 0.5 ml water) was

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then injected i.v. and further blood samples withdrawn after 1, 3, 6, 12, 24, and 48 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. Preliminary experiments showed that glucose loading did not affect the cardiovascular changes due to 8-OH-DPAT administration.

Plasma glucose, insulin and corticosterone measurements

Blood samples were collected in Eppendorf tubes through the arterial cannula and kept at 4°C before centrifugation (8800g for 3 min). Aliquots (10 µl) of the plasma were used to determine glucose concentrations by use of an automatic glucose analyser (Ektachem, Kodak). The remainder of the plasma was stored at -20°C for subsequent insulin and corticosterone assay. Insulin was determined, by use of the radioimmunoassays described by Hales & Randle (1963). Corticosterone was determined by a modification of a radioimmunoassay described by Abraham (1977). Briefly, 20 µl of plasma were diluted 500 fold with a phosphate buffer containing 0.2% gelatin. Diluted samples (500 µl) and standards were heated at 98°C for 10 min in order to denature corticosterone-binding rat plasma proteins. After the samples had cooled to room temperature, [³H]-corticosterone (100 µl, about 10,000 c.p.m.) and anti-corticosterone antibody (100 µl) were successively added. The antibody concentration was selected to produce 50% of maximal binding. After an incubation at 4°C for 2 h, separation of free corticosterone from antibody bound corticosterone was achieved by use of 200 µl of coated charcoal suspension (0.6%). Following centrifugation (2500 r.p.m. for 15 min), supernatants containing bound corticosterone were collected and counted in a β-counter.

Adrenal tissue catecholamine measurements

At the end of the experiment, adrenal glands were removed and stored at -180°C and subsequently, tissue adrenaline, noradrenaline and dopamine concentrations were measured by reverse phase high performance liquid chromatography and electrochemical detection according to the method of Kempf & Mandel (1981).

Drugs and solutions

(±)-8-OH-DPAT hydrogen bromide (Research Biochemicals Inc.) was dissolved in 0.9% (w/v) NaCl for *in vivo* experiments. Human [¹²⁵I]-insulin (Amersham International) and anti-insulin antiserum raised in guinea-pigs were from Medipro (Basel, Switzerland). Anticorticosterone antibody (Radioassay Systems Laboratories, Inc., Carson, Ca, U.S.A.) and tritiated corticosterone were from New England Nuclear (Regensdorf, Switzerland).

Calculations and statistics

Data have been expressed as means ± s.e.mean. Statistical analysis of data was performed by use of unpaired Student's *t* test and were considered significant at the *P* < 0.05 level. Positive incremental areas under the curve for plasma insulin and glucose concentration versus the time (AUC) was determined using the trapezoidal rule (Le Floch *et al.*, 1990).

Results

Conscious sham-adrenomedullated rats

In sham-operated animals, 8-OH-DPAT (150 µg kg⁻¹ i.v.) significantly increased basal plasma glucose concentrations after 15 min (Figure 1a), without a change in basal plasma insulin (Figure 1b). Following an intravenous injection of glucose (IVGTT) to 8-OH-DPAT-treated animals, the stimulated

Figure 1 Effects of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) on plasma glucose, insulin, blood pressure (BP) and heart rate (HR) in conscious sham-operated and adrenomedullated Sprague-Dawley rats. Basal plasma glucose and insulin concentrations were determined before 8-OH-DPAT (150 µg kg⁻¹, i.v.) injections and 15 min later. (a) Maximal changes in basal plasma glucose after 8-OH-DPAT. Basal plasma glucose levels were 137 ± 4 and 137 ± 9 mg dl⁻¹, *n* = 6, respectively in sham-operated and adrenomedullated animals. (b) Maximal changes in basal plasma insulin after 8-OH-DPAT. Basal plasma insulin levels were 49 ± 5 and 41 ± 5 µu ml⁻¹ respectively for sham-operated and adrenomedullated animals, *n* = 6. (c) Maximal changes in BP during the transient vasoconstriction and the sustained hypotension phases. Baseline values were respectively 109 ± 2 mmHg, *n* = 8 and 101 ± 4 mmHg, *n* = 6 for sham-operated and adrenomedullated animals. (d) Maximal changes in HR following 8-OH-DPAT administration. Baseline values were 417 ± 14 and 433 ± 9 respectively for sham-operated and adrenomedullated animals. Results are represented as mean with s.e.mean of changes shown by vertical bars. Solid columns: sham-operated animals; open columns: adrenomedullated animals. For further experimental details, see text. **P* < 0.05, value significantly different from the corresponding control value.

plasma insulin levels were reduced in association with an increase in plasma glucose levels. When both the first 15 min of 8-OH-DPAT-induced hyperglycaemia and the IVGTT were taken into account (0–63 min), a significant decrease in AUC for plasma insulin was found in the 8-OH-DPAT-treated group whereas the plasma glucose was increased by 41%, although the increase did not reach statistical significance due to the variation (Table 1). As expected, 8-OH-DPAT at the same dose produced sustained falls in both BP and HR which were preceded by transient (< 5 min) increases in BP (Figure 1c and d). Control saline injections did not produce any change of the above parameters (data not shown).

Conscious adrenomedullated rats

In contrast to sham-operated animals, 8-OH-DPAT did not modify plasma levels of glucose or insulin either under basal

Table 1 Effect of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) on areas under the curve (AUC) for basal and intravenous glucose tolerance test (IVGTT)-stimulated plasma glucose and insulin concentrations versus time

	AUC glucose		AUC insulin	
	Control	8-OH-DPAT	Control	8-OH-DPAT
Sham-operated (0–63 min)	3012 ± 388	5082 ± 1035	3626 ± 525	2310 ± 198*
Adrenodemedullated (0–63 min)	1510 ± 151	2004 ± 190	4023 ± 804	4250 ± 555

Values are expressed in $\mu\text{mol l}^{-1}$ and mg dl^{-1} respectively for plasma insulin and glucose. * $P < 0.05$, value significantly different from the corresponding control value. Areas under the curve (AUC) were calculated by use of the trapezoidal rule. Values determined for 63 min take into account both the effect on the basal levels during the first 15 min and the effects on the intravenous glucose tolerance test (IVGTT) during the 48 min period.

conditions or during the IVGTT in adrenodemedullated rats (Figure 1a; Table 1). 8-OH-DPAT produced similar falls in both BP and HR as compared to sham-operated animals (Figure 1c and d) that were, however, not preceded by the initial pressor response observed in sham-operated animals.

Assessment of adrenodemedullation

Adrenaline, noradrenaline and dopamine levels were respectively 426 ± 23 , 87 ± 7 and $12 \pm 1 \mu\text{g g}^{-1}$ of tissue in sham-operated animals and significantly reduced at 14 ± 11 , 3 ± 3 and $1 \pm 1 \mu\text{g g}^{-1}$ of tissue in adrenodemedullated animals. There were no significant differences between animals receiving 8-OH-DPAT or saline in either group (data not illustrated). Basal plasma corticosterone concentrations in sham-operated animals were significantly ($P < 0.05$) greater $57.7 \pm 5.1 \mu\text{g dl}^{-1}$, $n = 11$ than in adrenodemedullated animals $33.6 \pm 3.5 \mu\text{g dl}^{-1}$, $n = 10$. No statistically significant increases in corticosterone concentrations were observed 15 and 27 min after 8-OH-DPAT administration in either group of animals (data not illustrated).

Discussion

The major conclusion that emerges from this study is that the hyperglycaemic response of 8-OH-DPAT, its inhibitory action on glucose-stimulated insulin release and the transient vasoconstrictor effect are mediated by the medulla of the adrenal gland. The extent of adrenodemedullation was assessed by the determination of catecholamines in the adrenal gland tissue. A 95% reduction in noradrenaline, adrenaline and dopamine indicates nearly a complete adrenodemedullation and suggests a role for the medullary catecholamines in the metabolic and vasoconstrictor effects of 8-OH-DPAT. The fact that 8-OH-DPAT (Bagdy *et al.*, 1989; Chaouloff *et al.*, 1990a) increases plasma adrenaline and that selective α_2 - and β_2 -adrenoceptor antagonists (Chaouloff & Jeanrenaud, 1987; Chaouloff *et al.*, 1990a) suppress 8-OH-DPAT-mediated hyperglycaemia supports the involvement of adrenal catecholamines in the metabolic effects of 8-OH-DPAT. Moreover, the fact that the plasma glucose levels are significantly increased in sham-operated rats following 8-OH-DPAT administration, without significant increase in corticosterone, suggests that the hyperglycaemia is not a consequence of corticosterone release in the present study. The adrenaline-releasing effects of 8-OH-DPAT are antagonized by the mixed 5-HT_{1A}- β -adrenoceptor antagonist, (–)-pindolol, but not by selective β_1 - or β_2 -adrenoceptor antagonists (Chaouloff *et al.*, 1990a). Moreover, adrenaline release and hyperglycaemia is induced by several other selective 5-HT_{1A}-receptor agonists such as buspirone, ipsapirone (Chaouloff *et al.*, 1990b) and flesinoxan (Mir *et al.*, unpublished observations), thus implicating a key role for the 5-HT_{1A}-receptors in the regulation of adrenaline release and glucose metabolism.

8-OH-DPAT has been shown to increase plasma levels of corticosterone in the rat (Koenig *et al.*, 1987; Aulakh *et al.*,

1988; Chaouloff *et al.*, 1990c), which can be considered as another potential contributory factor to the 8-OH-DPAT-induced hyperglycaemia. However, the nature and extent of the involvement of corticosterone in this respect is not clearly established (Chaouloff *et al.*, 1990c). In the present study no significant stimulation of corticosterone levels was observed 15 min following 8-OH-DPAT administration. This lack of stimulation appears unlikely to be due to a non-functional cortex since in both adrenodemedullated and sham-operated animals corticosterone production was present.

With regard to the cardiovascular effects of 8-OH-DPAT, the transient vasoconstriction in response to 8-OH-DPAT, preceding the falls in BP and HR is abolished in adrenodemedullated animals. This finding, confirms a previous observation in adrenalectomized animals (Bouhelal & Mir, 1990), and together with the observations that 8-OH-DPAT increases plasma catecholamine levels (Bagdy *et al.*, 1989; Chaouloff *et al.*, 1990a) and that the pressor response is attenuated by prazosin (Gradin *et al.*, 1985), supports the involvement of catecholamines in the pressor response to 8-OH-DPAT. 8-OH-DPAT also produces a small transient pressor response in pithed rats (Fozard *et al.*, 1987; Bouhelal *et al.*, 1990). This action is unlikely to be due to catecholamine release, since Chaouloff *et al.* (1990d) have shown that pentobarbitone anaesthesia completely suppresses the adrenaline release which normally occurs in conscious animals. Rather, a direct vascular action in response to a bolus intravenous injection is a possible mechanism. The fact that 8-OH-DPAT produces similar hypotensive and bradycardic effects in sham and adrenodemedullated rats confirms previous observations in adrenalectomized rats (Bouhelal & Mir, 1990), and lends further support to the difference in mechanisms in the hypotensive and bradycardic effects *vis-a-vis* the vasoconstrictor and metabolic effects. Paradoxically, the metabolic changes (hyperglycaemia and hypoinsulinaemia) would generally be expected to arise from sympathoexcitation and/or a decrease in vagal tone (Ahren *et al.*, 1986), which is the opposite of what has been established to be the basis of the 8-OH-DPAT effects on the cardiovascular system (see Introduction). Thus it appears that 8-OH-DPAT exerts a differential action on the autonomic nervous system with respect to its metabolic and major cardiovascular effects (hypotension and bradycardia). In Figure 2, a tentative model to explain the mechanisms of 5-HT_{1A}-mediated metabolic and cardiovascular effects is illustrated. 5-HT and 8-OH-DPAT stimulate the release of corticotrophin-releasing factor (CRF) (Jones *et al.*, 1976; Calogero *et al.*, 1989). In addition to its well known ACTH-releasing activity, CRF is also known to stimulate directly sympathetic tone to the adrenal medulla via a central site of action (for review see Fisher, 1989), manifested by increases in efferent adrenal nerve activity, plasma adrenaline concentrations and an increase in BP (Brown & Fisher, 1983; Brown *et al.*, 1985). Hyperglycaemia has also been observed after intracerebroventricular injections of CRF in the rat and the dog (Brown *et al.*, 1982). The recent observations that ganglionic nicotine receptor blockade with hexamethonium decreases the 8-OH-DPAT-induced increases in plasma adrenaline and

Figure 2 Tentative model for 5-hydroxytryptamine_{1A} (5-HT_{1A}) - receptor-mediated metabolic and cardiovascular effects in the rat. CRF: corticotropin-releasing factor; ACTH: adreno-corticotrophic hormone; - : decrease; + : increase; CNS: central nervous system. For further details see Discussion.

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glucose (Chaouloff *et al.*, 1990c) would also be consistent with a centrally mediated sympatho-adrenal stimulation. Therefore, the possibility that 8-OH-DPAT-mediated catecholamine release, which would result in hyperglycaemia and hypo-insulinaemia, is triggered by CRF deserves further attention.

Taken together, these data strongly suggest that 8-OH-DPAT exerts its metabolic and transient pressor effect primarily via an action on the adrenal medulla. The data further confirm the differences in the mechanisms underlying the hypotensive and bradycardic actions of 8-OH-DPAT *vis-a-vis* the effects on glucose homeostasis. The major cardiovascular effects (hypotension and bradycardia) of 5-HT_{1A} agonists may thus be regarded as being mediated purely by neuronal pathways whereas the metabolic effects involve mixed neuronal and humoral mechanisms (see Figure 2).

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