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Marked increases in plasma catecholamine concentrations precede hypotension and bradycardia caused by 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) in conscious rats

GYORGY BAGDY, KATALIN SZEMEREDI*, DENNIS L. MURPHY, *Section on Clinical Neuropharmacology, Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, MD, USA* **Clinical Neuroscience Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA*

Abstract—Plasma noradrenaline and adrenaline responses to 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT), a selective putative 5-HT_{1A} receptor agonist, have been studied in conscious, freely moving rats. Intravenously administered 8-OH-DPAT caused dose-related and sustained increases in plasma noradrenaline (2-fold) and adrenaline (11-fold) concentrations. Neither metergoline pretreatment (0·5 mg kg^{-1} i.v.) nor splanchnicectomy had any effect on the noradrenaline and adrenaline elevation caused by 8-OH-DPAT (250 $\mu g\ kg^{-1}$ i.v.). The catecholamine responses peaked early but were still present during nadirs in blood pressure and heart rate. The discrepancy between plasma catecholamine and cardiovascular changes raises further questions about the mechanism of action of 8-OH-DPAT and supports other evidence suggesting a role for vagus stimulation in the cardiovascular effects caused by this drug.

8-Hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) has been shown to be a potent and selective centrally acting 5-hydroxytryptamine (5-HT) agonist (Hjorth et al 1982). In radioligand studies, 8-OH-DPAT shows high affinity and selectivity for the 5-HT_{1A} subtype of the 5-HT_1 receptor population (Middlemiss & Fozard 1983). In conscious rats, 8-OH-DPAT administration is associated with marked decreases in heart rate (HR) and blood pressure (BP) (Martin & Lis 1985), responses shown to be mainly centrally mediated (Gradin et al 1985; Fozard et al 1987). 8-OH-DPAT also produces a dose-related inhibition of sympathetic discharge in anaesthetized cats (McCall et al 1987). To determine whether 8-OH-DPAT's sympatholytic effects might result in changes in circulating catecholamines (CAs), we injected different doses of 8-OH-DPAT into conscious, freely moving rats and measured plasma noradrenaline and adrenaline concentrations.

Materials and methods

Male Sprague-Dawley rats, 280-350 g (Taconic Farms, Germantown, NY), were housed 5-6 per cage and maintained on a 12 h light–dark cycle. Food and water were freely available. Splanchnicectomized rats were obtained, 2–3 days after the surgery, from the same source. The rats were allowed 1–2 weeks to acclimate and/or recover from surgery.

At least 24 h before an experiment, polyethylene (PE-50) cannulae were implanted in the left femoral artery and vein under anaesthesia with 2% halothane in oxygen. Cannulae were exteriorized subcutaneously at the back of the neck. Dead space of the cannulae was approximately 150 μL. After cannulation, animals were housed separately, with food and water freely available. This system allowed us to obtain serial blood samples from conscious, unrestrained rats. Cannulae were flushed two times a day with 0·3 mL of a 0·9% saline solution containing 200 U mL^{-1} heparin (Hynson, Westcott and Dunning, Baltimore, MD).

Baseline blood samples were obtained 15-30 min before the drug or saline control was administered. 8-OH-DPAT (Research Biochemical, Inc., Wayland, MA) in doses ranging from 3·9 to 500 $\mu g\ kg^{-1}$ was dissolved in saline and metergoline (0·5 mg kg^{-1}, Farmitalia, Carlo Erba) in 1% ascorbic acid. Both drugs and control saline were administered intravenously into the femoral vein in a total volume of 0·5 mL kg^{-1} weight during 10 s.

Blood samples (0·35 mL) were collected by free flow via the intra-arterial cannula in heparinized polyethylene tubes. The sample volume was returned immediately to the animal as heparinized saline. Blood samples were refrigerated and centrifuged immediately, and aliquots (120 μL) of plasma were removed and stored frozen until simultaneous radioenzymatic assay of adrenaline and noradrenaline. HR and BP were recorded (Beckman R511A dynograph, 4-327-0 pressure transducer, and cardiotachometer coupler type 9857B) in the groups receiving 250 $\mu g\ kg^{-1}$ of 8-OH-DPAT or saline.

Plasma was stored at −80°C for no more than two weeks before assay. Thawed aliquots of plasma (120 μL) were mixed with 120 μL of 0·5 M perchloric acid containing 31·8 nM EGTA and centrifuged at 5000 *g* for 10 min at 4°C; the protein-free supernatants (150 μL) were used for the assay. The concentrations of noradrenaline and adrenaline were measured using the radioenzymatic assay described by Zukowska-Grojec et al (1985), adapted from the method previously reported by Weise & Kopin (1976). The sensitivity was 2 and 3 pg/tube for adrenaline and noradrenaline, respectively. Some plasma samples were also analysed using HPLC-EC (Eisenhofer et al 1986) to ensure that 8-OH-DPAT was not interfering with the assay; essentially identical results were obtained.

A repeated measures analysis of variance accompanied by single degree of freedom contrasts (GLM procedure, SAS Institute, Cary, NC) was used to compare the results of drug or saline administration. Where applicable, one-way analysis of variance and contrasts specified deductively were used to characterize further changes from baseline resulting from drug treatment. All data are given as means ± s.e.m.

Results

Serial blood samples obtained after administration of 8-OH-DPAT revealed marked, long-term increases in plasma adrenaline and noradrenaline concentrations, while saline administration had no significant effect (Fig. 1). Plasma CAs peaked at 10 min after the drug administration, while nadirs in BP and HR occurred at 30 and 45 min, respectively.

The two lowest doses of 8-OH-DPAT had no significant effect on adrenaline and noradrenaline concentrations, while higher doses of the drug (62·5, 250, and 500 $\mu g\ kg^{-1}$) caused large elevations in plasma adrenaline and smaller increases in plasma noradrenaline (Fig. 2).

Metergoline pretreatment (0·5 mg kg^{-1} i.v., 15 min before 8-OH-DPAT) or bilateral splanchnicectomy did not cause significant changes in CA responses to 250 $\mu g\ kg^{-1}$ 8-OH-DPAT (noradrenaline, 10 min: control, n = 6, +2·98 ± 0·28 nM, metergoline pretreatment, n = 6, +3·79 ± 0·59 nM, splanchnicectomy,

Correspondence to: G. Bagdy, Section on Clinical Neuropharmacology, Laboratory of Clinical Science, National Institute of Mental Health, NIH Clinical Center, 10-3D41, Bethesda, MD 20892, USA.

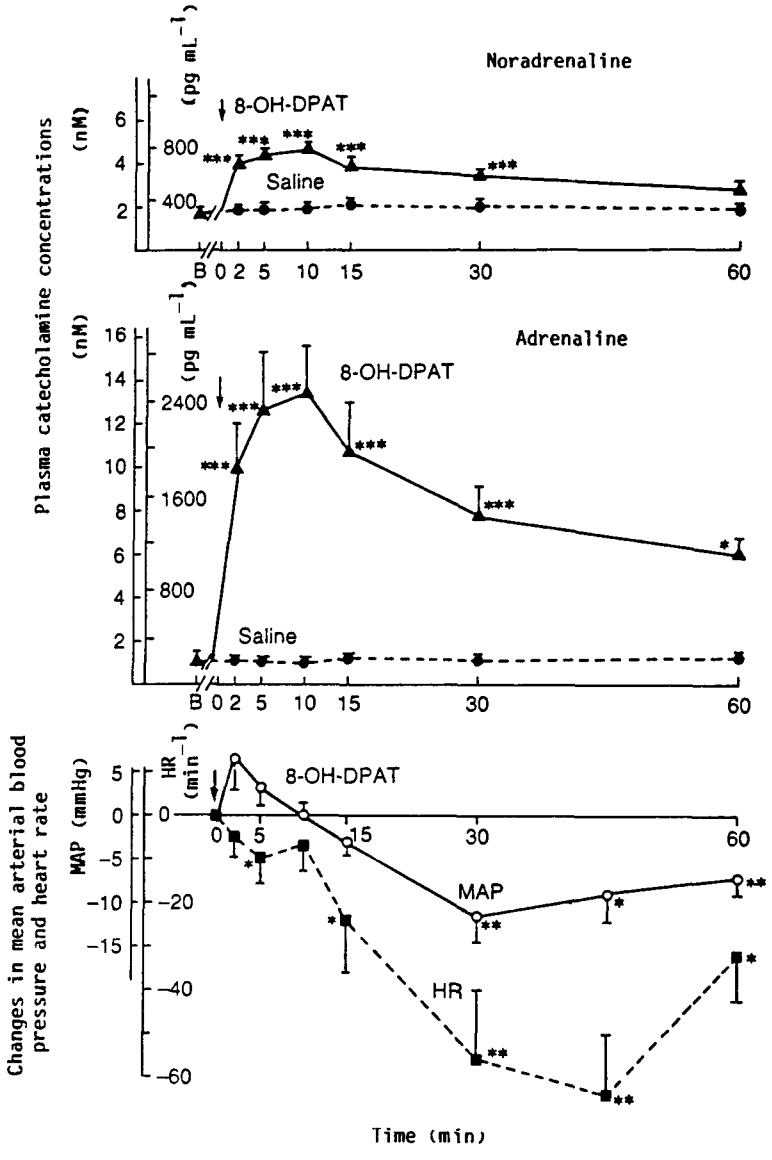

FIG. 1. Effect of 8-OH-DPAT (250 μg kg^{-1}; n = 6) and saline (n = 8) on plasma noradrenaline and adrenaline levels and parallel mean arterial blood pressure (MAP) and heart rate (HR) changes after 8-OH-DPAT over time. Significant differences in drug-induced changes from baseline compared to saline are denoted by * ($P < 0.05$); ** ($P < 0.01$); and *** ($P < 0.001$; one-way analysis of variance).

n = 7, +3·06 ± 0·46 nM; adrenaline, 10 min: control, n = 6, +12·1 ± 1·99 nM; metergoline pretreatment, n = 6, +11·79 ± 1·54 nM, splanchnicectomy, n = 7, +10·1 ± 1·66 nM).

Discussion

Earlier reports of hypotension, bradycardia, and decreased sympathetic nerve activity after administration of 8-OH-DPAT led us to anticipate that 8-OH-DPAT would cause decreases in plasma CAs. Following 8-OH-DPAT administration, however, we observed markedly increased plasma CA concentrations. An obvious explanation for this occurrence would be that these changes resulted from a reflex increase in sympathetic tone caused by a decrease in BP. However, the peaks in plasma CA responses occurred much earlier than the modest decrease in BP. Furthermore, during these CA peaks, HR was found to decrease.

Using the same doses of metergoline (0·5 mg kg^{-1}) and 8-OH-DPAT (250 μg kg^{-1}), Aulakh et al (1988) found that metergoline decreased plasma prolactin responses and increased temperature responses to 8-OH-DPAT. The lack of effect of metergoline on the plasma CA responses to 8-OH-DPAT suggests that a non-hydroxytryptaminergic action of 8-OH-DPAT may be involved. The greater adrenaline than noradrenaline release observed, combined with prior evidence that 8-OH-DPAT inhibits rather than activates sympathetic nerve discharge (McCall et al 1987) suggests 8-OH-DPAT may act directly on the adrenal gland and/or sympathetic nerve terminals to increase CA release. One possible mechanism is that 8-OH-DPAT might act like the α_2-antagonist, yohimbine, which increases plasma CAs in conscious rats (Graham et al 1980); 8-OH-DPAT has recently been reported to act as an α_2-adrenoceptor antagonist on guinea-pig neurons (Crist & Suprenant 1987).

It has not yet been determined how the increases in plasma adrenaline and noradrenaline relate to the hypotension and bradycardia produced by 8-OH-DPAT. However, it is possible that the apparent increases in central vagal tone reported by Gradin et al (1985) and Ramage & Fozard (1987) following 8-

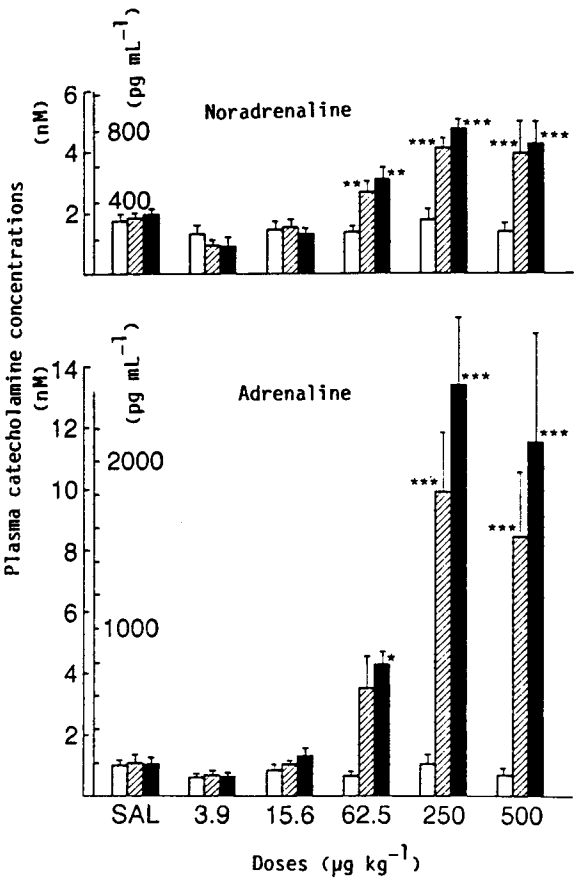

FIG. 2. Plasma noradrenaline and adrenaline concentrations (means ± s.e.m.) before injection (open column), and 2 min (hatched column) and 10 min (solid column) after i.v. injection of saline (SAL) and different doses of 8-OH-DPAT in conscious rats. Each group represents 5–7 animals. Significant differences in drug-induced changes from baseline compared to saline are denoted by * ($P < 0.05$); ** ($P < 0.01$); and, *** ($P < 0.001$; one-way analysis of variance).

OH-DPAT may help explain the concurrence of hypotension and reduced HR with increased CAs following 8-OH-DPAT administration. Furthermore, adrenaline stimulates vasodilator β_2-adrenoceptors as well as α-adrenoceptors. 8-OH-DPAT may also activate other vasodilator mechanisms not yet identified. A possible candidate for the vasodilator substance might be met-enkephalin, which is stored and secreted with adrenaline from the chromaffin cells of the adrenal medulla (Viveros et al 1979; Hexum et al 1980). Thus, our findings are consistent with studies suggesting increased vagal activity as a basis for the cardiovascular effects of 8-OH-DPAT and raised further questions regarding multiple mechanisms of action of this drug.

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