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Direct angiotensin II type 2 receptor stimulation decreases dopamine synthesis in the rat striatum

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ABSTRACT

A relationship between the central renin angiotensin system and the dopaminergic system has been described in the striatum. However, the role of the angiotensin II type 2 (AT_2) receptor in this interaction has not yet been established. The present study examined the outcome of direct AT_2 receptor stimulation on dopamine (DA) release and synthesis by means of the recently developed nonpeptide AT_2 receptor agonist, compound 21 (C21). The effects of AT_2 receptor agonism on the release of DA and its major metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) and on the activity of tyrosine hydroxylase (TH), the rate-limiting enzyme in the catecholamine biosynthesis, were investigated using *in vivo* microdialysis. Local administration of C21 (0.1 and 1 μM) resulted in a decrease of the extracellular DOPAC levels, whereas extracellular DA concentrations remained unaltered, suggesting a reduced synthesis of DA. This effect was mediated by the AT_2 receptor since it could be blocked by the AT_2 receptor antagonist PD123319 (1 μM). A similar effect was observed after local striatal (10 nM) as well as systemic (0.3 and 3 mg/kg i.p.) administration of the AT_1 receptor antagonist, candesartan. TH activity as assessed by accumulation of extracellular levels of L-DOPA after inhibition of amino acid decarboxylase with NSD1015, was also reduced after local administration of C21 (0.1 and 1 μM) and candesartan (10 nM). Together, these data suggest that AT_1 and AT_2 receptors in the striatum exert an opposite effect on the modulation of DA synthesis rather than DA release.

1. Introduction

It is widely accepted that the renin angiotensin system (RAS) modulates salt and water homeostasis and that angiotensin II (Ang II), its major effector peptide, exerts a hypertensive effect. Most of the classical peripheral actions of Ang II including vasoconstriction, facilitation of sympathetic transmission and renal water and salt retention, are mediated by the Ang II type 1 (AT_1) receptor although at least one other receptor subtype, the AT_2 receptor, has been described (Culman et al., 2002). AT_2 receptors are widely distributed in fetal tissue (Grady et al., 1991), but their expression is dramatically decreased after birth. Little is known about the physiological role of the AT_2 receptor, yet its activation is assumed to be associated with cell proliferation, cell differentiation, tissue regeneration, and even with apoptosis (Cote et al., 1999; de Gasparo et al., 2000; Laflamme et al., 1996; Li et al., 2005; Meffert et al., 1996; Stroth et al., 1998). Until recently, the study of the AT_2 receptor mediated effects was hindered as these functional experiments had to be performed either by treatment with Ang II under concomitant blockade of the AT_1 receptor or in genetically altered animals. A more elegant approach is to use the AT_2 selective peptide agonist CGP42112. However the use of CGP42112 was restricted due to the observation that this compound displays only partial agonist activity (Kaschina et al., 2008). Furthermore, peptides in general are very susceptible to enzymatic degradation in *in vivo* models. With this respect, an important breakthrough was achieved by the development of a selective and potent nonpeptide AT_2 receptor agonist, compound 21 (C21) (Wan et al., 2004).

In addition to the well-described peripheral RAS, there is now accumulating evidence for the presence of a central RAS (Fischer-Ferraro et al., 1971; Phillips and de Oliveira, 2008). Both in the periphery and the brain, the hypertensive actions of Ang II have been correlated with the regulation of catecholamine metabolism. Indeed, Ang II has been shown to modulate the synthesis, uptake and release of catecholamines in the sympathic neurons and the adrenal medulla as well as in central cardioregulatory areas such as the hypothalamus and the brainstem (Yu et al., 1996).

Interestingly, an Ang II-catecholaminergic interaction has also been described in the striatum, a brain region that is not involved in the regulation of blood pressure but in the control of movement. All the components of the RAS, including AT_1 and AT_2 receptors, have been detected in this brain nucleus (for review Mertens et al., 2009)

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and local administration of Ang II in the rat striatum enhanced the release of dopamine (DA). However, the mechanism involved in this release remains to be established (Brown et al., 1996; Mendelsohn et al., 1993; Stragier et al., 2004).

Until now, most studies have focused on the effect of Ang II via the AT_1 receptor on DA release and little attention was paid to the involvement of the AT_2 receptor in DA synthesis and release. In this context, the present study is the first to examine the role of the AT_2 receptor in DA release and synthesis in the striatum by means of compound 21 (C21). *In vivo* microdialysis was used to investigate the effects of AT_2 receptor stimulation on the release of DA and its major metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) and on the activity of tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis. The results suggest that the AT_2 receptor in the striatum is involved in the modulation of DA synthesis rather than DA release.

2. Methods

2.1. Animals

Animal experiments were carried out according to the national guidelines [Belgian guideline on the protection of laboratory animals (KB Nov 1993) and the Revised European Guideline (appendix E to ETS123)] on animal experimentation and were approved by the Ethical Committee for Animal Experiments of the Faculty of Medicine and Pharmacy of the Vrije Universiteit Brussel. All efforts were made to minimize animal suffering and the minimal number of animals necessary to produce reliable scientific data was used.

2.2. Stereotaxic implantation of the microdialysis probe

Male albino Wistar rats (Charles River, Brussels, Belgium) weighing 275–300 g were used in this study. Rats were first anaesthetized with a mixture of ketamine (90.5 mg/kg i.p.; Ketamine 1000 Ceva®, Ceva Sante Animale, Brussels, Belgium) and diazepam (4.5 mg/kg i.p.; Valium®, Roche, Brussels, Belgium) and placed on a Kopf stereotaxic frame (ear bars positioned symmetrically). The skull was exposed and a burr hole was drilled to implant a guide cannula (MAB 2/6/9.14.IC, Microbiotech/se AB, Stockholm, Sweden) positioned 3 mm above the left dorsal striatum according to the atlas of Paxinos and Watson (1998) (coordinates relative to bregma: L: −2.4, A: +1.2 and V: +2.8). The animals received ketoprofen (4 mg/kg i.p.; Ketofen®, Merial, Brussels, Belgium) as analgesic. After surgery, a microdialysis probe (MAB 6.14.3, Microbiotech/se AB) with a membrane length of 3 mm and a molecular cut-off value of 15 kDa was introduced via the cannula. The probe was perfused with modified Ringer's solution containing 147 mM NaCl, 4 mM KCl and 1.1 mM $CaCl_2$ at a constant flow rate of 2 μl/min using a microdialysis pump (CMA 100; CMA Microdialysis, Solna, Sweden).

Animals were allowed to recover from surgery overnight and dialysate collection was started the next day.

2.3. In vivo microdialysis experiments

Samples were collected every 20 min, yielding 40 μl dialysates, in vials containing 10 μl of a filtered antioxidant mixture (0.1 M acetic acid, 3.3 mM L-cysteine, 0.27 mM Na_2 EDTA, 12.5 μM ascorbic acid).

For the experiments in which the effects on *the release of DA and DOPAC* were studied, four to six dialysate samples were collected before any pharmacological manipulation was performed. The mean of these neurotransmitter dialysate concentrations was taken as baseline value at time zero. After the baseline samples, the drugs dissolved in modified Ringer's solution were locally administered via the probe. In a first set of experiments, compound 21 [*N*-Butyloxycarbonyl-3-(4-imidazol-1-ylmethylphenyl)-5-isobutylthiophene-2-sulfonamide, C21] was perfused for 2 h after 6 baseline samples. Two concentrations of the drug were tested (0.1 and 1 μM). Another set of experiments was carried out in order to investigate the effect of an AT_1 receptor antagonist [2-ethoxy-1-[(4-[2-(2*H*-1,2,3,4-tetrazol-5-yl)phenyl] phenyl) methyl]-1*H*-1,3-benzodiazole-6-carboxylic acid, candesartan, 10 nM] and an AT_2 receptor antagonist [S-(+)-1-([(4-dimethylamino)-3-methylphenyl]methyl)-5-(diphenyl-acetyl)-4,5,6,7-tetrahydro-1*H*-amidazo(4,5-c) pyridine-6-carboxylic acid, PD123319, 1 μM] on the C21-induced changes. Four baseline samples were collected, followed by the perfusion of the antagonist alone for 2 h. Then, C21 (0.1 and 1 μM) was administered together with the antagonist for another 2 h.

The pharmacological manipulations were always followed by a perfusion with modified Ringer's solution for another 60 min.

In order to compare the effects of a local perfusion of the compounds with those of a systemic administration, candesartan was injected intraperitoneally. In this set of experiments, candesartan (0.3 and 3 mg/kg) was injected after six baseline samples and collection of dialysate was continued during 240 min. Candesartan has been reported to cross the blood brain barrier and to block AT_1 receptors in the brain (Song et al., 1999). With respect to C21, until now there is no evidence that this drug crosses the blood brain barrier after systemic administration. Preliminary experiments in which the concentration of C21 in striatal dialysates was determined with liquid chromatography suggest that C21 is not able to reach the striatum after systemic application, at least not in a consistent, reproducible way (data not shown). This hypothesis is further supported by the observation that striatal DA and DOPAC levels remained unchanged after the systemic administration of C21 (50 mg/kg i.p.) (data not shown). Therefore, systemic administration of C21 was not further considered in this study.

C21 was kindly provided by Vicore Pharma (Göteborg, Sweden), PD123319 was purchased from Sigma (St. Louis, MO, USA), whereas candesartan was a gift from AstraZeneca (Mölndal, Sweden). The concentrations of C21 were chosen according to the pK_i-value (Wan et al., 2004). The concentrations of candesartan and PD123319 were the same as previously used in the laboratory (Stragier et al., 2004). The dose of candesartan for systemic administration was based on literature findings (Gohlke et al., 2002).

To assess the *TH activity*, the method described by Westerink et al. (1990) was used, with slight modifications. After two baseline samples, an aromatic amino acid decarboxylase inhibitor (m-hydroxybenzylhydrazine, NSD1015, 10 μM, Sigma) was added to the modified Ringer's solution which resulted in the accumulation of L-3,4-dihydroxyphenylalanine (L-DOPA) in the dialysates. Stable L-DOPA levels were obtained 2 h after the initiation of NSD1015 administration. Once a stable L-DOPA concentration was reached, four samples were collected and the mean of these L-DOPA concentrations was used as baseline value at time zero. Afterwards, pharmacological manipulations were performed with C21 (0.1 and 1 μM), candesartan (10 nM) and PD123319 (1 μM) during 80 min. All compounds were dissolved in modified Ringer's solution containing 10 μM NSD1015. In order to verify if the AT_2 receptor was involved in the C21-induced effects, the drug (1 μM) was co-perfused with the AT_2 receptor antagonist, PD123319 (1 μM).

2.4. Chromatographic assay

For the determination of the DA, DOPAC and L-DOPA concentrations in the dialysates, three slightly different liquid chromatography (LC) assays were used.

For the DA analysis, 10 μl of dialysate with antioxidant solution was injected on an LC system that consisted of an isocratic pump (Bioanalytical systems, Indianapolis, IN, USA) delivering the mobile phase to a microbore column (C8 column: 5 μm, 100 × 1.0 mm, Bioanalytical Systems) at a flow rate of 100 μl/min. The mobile phase contained 0.1 M sodium acetate trihydrate (Merck, Darmstadt, Germany), 20 mM citric acid monohydrate (Merck), 2 mM decane sulfonic acid (Sigma) and 0.5 mM Na_2 EDTA (Merck) adjusted to pH 5.5. Acetonitrile (28 ml) was added to 200 ml of this buffer solution as organic modifier. The electrochemical detection (Decade, Antec, Leiden, The Netherlands) potential was +450 mV versus the reference electrode (Ag/AgCl).

In order to determine the extracellular DOPAC concentrations, 10 μl of the dialysate with antioxidant solution, was diluted 10 times in a mixture of antioxidant solution and modified Ringer's solution (1:4 v/v). Twenty microliter of the diluted sample was injected and analyzed directly on a narrowbore (C18 column: 15 μm, 150 × 2.1 mm; Alltima, Grace, Lokeren, Belgium) LC system. The mobile phase consisted of 0.1 M sodium acetate trihydrate (Merck, Darmstadt, Germany), 20 mM citric acid monohydrate (Merck), 1 mM 1-octane sulfonic acid (Sigma), 0.1 mM Na_2 EDTA (Merck) and 1 mM dibutylamine (Sigma), adjusted to pH 3.7. Methanol 3% (v/v) was added as organic modifier. The flow rate was set at 0.3 ml/min. The electrochemical detection (Antec) potential was +700 mV versus the reference electrode (Ag/AgCl). Sensitivity was set at 2 nA full scale. L-DOPA analysis was performed on the same LC system. However, the pH of the buffer was set at 2.9 and no organic modifier was added (Sarre et al., 1992).

All samples were injected via a high precision auto-injector equipped with a cooling system (Kontron, San Diego, CA, USA). The integration of the chromatograms was done with the Data Apex Clarity software programme (Antec).

2.5. Statistical analysis

No corrections were made for probe recovery across the dialysis membrane. Therefore, the reported extracellular concentrations are actually dialysate concentrations. The striatal extracellular concentrations of DA and DOPAC in baseline conditions were expressed as respectively nM and μM (mean ± SEM). Extracellular DA and DOPAC levels after the pharmacological manipulation were calculated as percentages (mean ± SEM) of the mean baseline value, expressed as 100%. As a plateau phase in the effect was already reached 20 min after the start of the perfusion with the different compounds, the average DA or DOPAC levels of the 5 consecutive samples during drug perfusion was calculated and used for statistical analysis. For the NSD1015-induced L-DOPA levels, this average was calculated only for the 3 consecutive samples since perfusion with the compounds was continued for 60 instead of 100 min. An unpaired *t*-test was used to analyze the effects of the different treatments on the baseline values of DA, DOPAC and NSD1015-induced L-DOPA levels and to compare the effects between different treatments. For statistical analysis of the effect of systemic administration of candesartan, one-way analysis of