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Ability of angiotensin II to modulate striatal dopamine release via the AT_1 receptor *in vitro* and *in vivo*

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1 The ability of angiotensin II to modulate dopamine release from rat striatal slices *in vitro* and in the intact rat striatum *in vivo* was assessed by the microdialysis technique.

2 In slices of rat striatum, angiotensin II (0.1–1.0 μM) induced a concentration-related increase in endogenous dopamine release which was maximal (approximately 250% above basal levels) within the first 2–4 min of agonist application and subsequently declined to near basal values. The angiotensin II-induced increase in dopamine release was Ca^{2+}-dependent and was completely antagonized by the selective AT_1 receptor antagonist, losartan (1.0 μM). In contrast, the AT_2 receptor antagonist, PD123177 (1.0 μM) failed to modify the angiotensin II-induced response. Neither antagonist alone modified basal dopamine release from striatal slices.

3 In freely moving rats, angiotensin II (1.0–10 μM; administered via the microdialysis probe) induced a concentration-related increase in extracellular levels of dopamine which was maximal (approximately 150% above basal levels) within 20–40 min of agonist application and subsequently declined. The angiotensin II (10 μM)-induced increase in extracellular levels of dopamine was completely antagonized by the AT_1 receptor antagonist, losartan (0.1–1.0 μM; administered via the microdialysis probe) but not by the AT_2 receptor antagonist, PD123177 (1.0 μM; administered via the microdialysis probe). Neither antagonist alone modified basal extracellular levels of dopamine.

4 Homogenate radioligand binding studies with [^{125}I]-angiotensin II (0.1 nM) identified relatively low levels of specific binding sites in rat striatal homogenates compared to homogenates of pyriform cortex (51.3±9.2 and 651.3±55.1 fmol g^{-1} wet weight, respectively, mean±s.e.mean, $n=3$; non-specific binding defined by unlabelled angiotensin II). The majority of the specific [^{125}I]-angiotensin II (0.1 nM) binding in the striatal and pyriform cortex homogenates was sensitive to the selective AT_1 receptor antagonist, losartan (1.0 μM).

5 In conclusion, the present study provides direct evidence that angiotensin II acting via the AT_1 receptor subtype facilitates the release of dopamine in the rat striatum *in vitro* and *in vivo*. This receptor-mediated response may account for the modulation of dopamine-mediated behavioural responses by antagonists of the AT_1 receptor and inhibitors of angiotensin converting enzyme.

Keywords: Angiotensin II; AT_1 receptor; dopamine release; *in vivo* microdialysis; striatum

Introduction

Both pharmacological and molecular data indicate the presence of at least two receptors for the octapeptide angiotensin II (AT_1 and AT_2; Chiu *et al.*, 1994; Inagami *et al.*, 1994). Both these AT receptor subtypes have been shown to mediate responses evoked by angiotensin II although to date, the majority of the 'classical' physiological responses evoked by angiotensin II appear to be mediated via the AT_1 receptor (e.g. Brown & Sernia, 1994; Dzau *et al.*, 1994). Substantial evidence indicates that the brain possesses a distinct angiotensin system (for review see Lippoldt *et al.*, 1995), pharmacological manipulation of which may modulate a number of processes co-ordinated by the CNS such as drinking behaviour (for review see Steckelings *et al.*, 1992), hormone release (for review see Steele, 1992), anxiety (Barnes *et al.*, 1990; 1991a; Kaiser *et al.*, 1992; Tsuda *et al.*, 1992), cognition (Mergan & Routlenberg, 1977; Barnes *et al.*, 1991a; 1991b; 1992a; Dennes & Barnes, 1993; Wayner *et al.*, 1993), locomotor activity (Georgiev & Kambourova, 1991; Banks *et al.*, 1992) and stereotypy (Georgiev, 1990; Banks & Dourish, 1991).

The neurochemical mechanisms which underlie the CNS-mediated responses following manipulation of the angiotensin system are poorly understood. It has been appreciated for some time that angiotensin II modulates catecholamine neurotransmitter release in the peripheral nervous system (for reviews see Story & Ziogas, 1987; Reid, 1992) and it is therefore of interest that growing evidence implicates a similar ability of angiotensin II to facilitate the neurotransmission of catecholamines in the CNS (Huang *et al.*, 1987; Finocchiaro *et al.*, 1990; Barnes *et al.*, 1991a; Qadri *et al.*, 1991; Dwoskin *et al.*, 1992; Stadler *et al.*, 1992; Mendelsohn *et al.*, 1993; Steward *et al.*, 1993). Given the established role of the nigro-striatal dopamine system in the control of movement, the demonstration that angiotensin II enhances the release of dopamine in the rat striatum may provide a neurochemical mechanisms underlying the modulation of locomotor activity and other dopamine-mediate behaviours that have been shown to be susceptible to pharmacological manipulation following interaction with the angiotensin system (see above). In the present study, we investigate the ability of angiotensin II to modulate the release of striatal dopamine *in vitro* and *in vivo* and characterize the receptor, using AT receptor subtype-selective non-peptide antagonists. A preliminary account of the *in vitro* work has been presented to the British Pharmacological Society (Brown & Barnes, 1993).

Methods

Animals

Female Wistar rats (190–310 g; University of Birmingham) were housed in groups of 5–6 in a temperature and humidity-

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controlled environment and were given free access to food and water.

Preparation of striatal slices

Rats were killed by cervical dislocation. The dissected striata were chopped (400 × 400 μm × thickness of the striatum; McIlwain tissue chopper) and the slices loaded into perfusion chambers and perfused with gassed (95/5 O_2/CO_2) Krebs buffer (mM: NaCl 120, KCl 4.75, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 11) plus the dopamine precursor tyrosine (50 μM), GBR12909 (1.0 μM) and (−)-sulpiride (1.0 μM) for 120 min before the continuous collection of 2 min perfusate fractions. The selective dopamine uptake blocker, GBR12909 and the selective dopamine D_2 receptor antagonist (−)-sulpiride were included in the perfusing Krebs buffer to maximize dopamine levels in the perfusate by inhibiting the re-uptake of dopamine into the neurone and preventing the inhibitory presynaptic dopamine D_2 receptor from 'dampening' any drug-induced facilitation of dopamine release by increasing the tone on the dopamine D_2 autoreceptor. Protein content of the perfused striatal slices was assayed by the method of Bradford (1976) with bovine serum albumin used as the standard.

Stereotaxic implantation of chronic indwelling guide cannulae

Rats were anaesthetized with ketamine (60 mg kg^{-1}, i.p.) and medetomidine (250 μg kg^{-1}, i.p.) before the stereotaxic insertion of 5 mm chronically indwelling guide cannulae (19 gauge stainless steel tubing; the tips of the indwelling guide cannulae were in the cerebral cortex overlying the striatum; final microdialysis probe tip location, A + 0.2, V − 7.5, L − 2.5 relative to Bregma; Paxinos & Watson, 1986) which were secured to the skull with screws and dental cement. The guide cannulae were kept patent with stylets which protruded 0.5 mm out of the cannula.

Microdialysis probe construction, implantation and collection of dialysates

Microdialysis probes were constructed 'in house' as described previously (Barnes *et al.*, 1992b). Briefly, stainless steel 'bodies' (11 mm, 23 guage, Coopers Needle Work Ltd) and 'collars' (5 mm, 26 guage, Coopers Needle Work Ltd) were fixed into a perspex holder. Dialysis membrane (external/internal diameter 310/322 μM, molecular weight cut off 40,000; AN69, Hospal Medical) was glued to the tip of the stainless steel 'body' and the end sealed with eopxy resin (Araldite) leaving 4 mm in length exposed to the brain area. A silica glass fibre (external/internal diameter 140/74 μm, Scientific Glass Engineering PTY Ltd) guided artificial cerebrospinal fluid (aCSF; mM: NaCl 126, $NaHCO_3$ 27.4, KCl 2.4, KH_2PO_4 0.5, $CaCl_2$ 1.1, $MgCl_2$ 1.3, $NaHPO_4$ 0.49, glucose 7.0; pH 7.4) to the tip of the microdialysis probe, which subsequently eluted from the probe (via coiled polypropylene tubing) following passage between the outer surface of the silica glass fibre and the inner surface of the dialysis membrane.

At least 14 days after stereotaxic implantation of the guide cannulae, rats were immobilized by a soft-cloth wrapping technique and the microdialysis probe was gently implanted into the striatum and secured with cyanoacrylate adhesive (Loctite Superglue 3). The rat was placed in a single animal cage (with free access to food and water) overnight before being transferred to the test cage (40 × 25 × 20 cm; length, width, height) where the animal also had free access to food and water. Following a 60 min period for the rat to habituate to the test box, the microdialysis probe was connected, via polypropylene tubing, to a microdialysis pump (CMA 100, Carnegie) and was perfused at a constant rate of 2.0 μl min^{-1} with aCSF. The dialysate samples were collected every 20 min. All samples were analysed immediately for levels of dopamine by high performance liquid chromatography (h.p.l.c.) with electrochemical detection (e..c.d.) and drug/vehicle application commenced following the establishment of a stable baseline. At the end of the experiment, microdialysis probe placement was visually verified by coronal slicing of the brain using a cryostat. Data from animals where the microdialysis probes were not correctly located within the striatum were not included in the present study.

H.p.l.c.–e.c.d. system for the quantification of dopamine

For the determination of dopamine levels in perfusates (*in vitro* release experiments) and dialysates (*in vivo* release experiments), the h.p.l.c.–e.c.d. system comprised an isocratic pump (BAS PM-80 or Gynkotek 300) which was connected to an analytical column (Spherisorb 5 ODS-2; 150 × 4.6 mm; HPLC Technology) via a Rheodyne injector. The effluent from the analytical column was passed into an electrochemical detector (BAS LC-4C or Antec VT-03; glassy carbon working electrode set at +700 mV versus a Ag/AgCl reference electrode). The output from the electrode was monitored with a recording/plotting integrator (JCL6000 or MacIntegrator). The h.p.l.c.–e.c.d. system, with the exception of the integrator, was maintained at a constant temperature of 4°C inside a glass-fronted cool cabinet. The optimised mobile phase (methanol 10% v/v, sodium dihydrogen phosphate 0.12 M, octane sulphonic acid 0.55 mM; pH 5.4; slight adjustments to the pH, methanol concentration and/or octane sulphonic acid concentration were made to overcome variations in the chromatography) was delivered to the analytical column at a rate of 0.9–1.0 ml min^{-1}. Injections of external standards were made in order to identify and calibrate the peaks resulting from the injection of the perfusates and dialysates.

[^{125}I]-angiotensin II homogenate binding

Individual rat brain tissues (parietal cortex, pyriform cortex, striatum) were homogenized (Polytron setting 7, 10 s) in ice-cold incubation buffer (mM: NaCl 150, KH_2PO_4 50, $MgCl_2$ 10, ethylene glycol-*bis* (β-amino-ethyl-ether)*N,N,N',N'*-tetra-acetic acid (EGTA) 5 and 0.5% w/v bovine serum albumin, final pH 7.4) and centrifuged (48000 *g*, 4°C, 10 min). The pellet was gently resuspended in incubation buffer and recentrifuged (48000 *g*, 4°C, 10 min). The binding homogenate was formed by gentle resuspension of the pellet in incubation buffer at a final concentration of 100 mg original wet weight ml^{-1}. The preparation of the binding homogenate was performed immediately prior to assay. For [^{125}I]-angiotensin II binding, test-tubes in triplicate contained 50 μl competing compound (or vehicle; incubation buffer) and 50 μl [^{125}I]-angiotensin II (final concentration 0.09–0.11 nM); 100 μl brain homogenate was added to initiate binding which was allowed to proceed for 60 min at 25°C before termination by rapid filtration through pre-wet (0.1% v/v polyethyleneimine in incubation buffer) GF/B filters followed by washing (16 s) with ice-cold incubation buffer. Radioactivity was quantified in a gamma-counter.

Drugs

Angiotensin II (Sigma), GBR 12909 (1-[2-[*bis*(4-fluorophenyl) methoxy]ethyl]-4-[3-phenylpropyl]piperazine dihydrochloride; Research Biochemicals Inc.), losartan (DuP753; DuPont Merck Pharmaceuticals), PD123177 (1-(4-amino-3-methylphenyl)-methyl-5-diphenyl-acetyl-4, 5, 6, 7-tetrahydro-1*H*-imidazo [4, 5-C] pyridine-6-carboxylic acid; DuPont Merck Pharmaceuticals), tetrodotoxin (Sigma) and tyrosine (Sigma) were dissolved in a minimum volume of distilled water and diluted to volume in Krebs buffer, aCSF or radioligand binding incubation buffer, as appropriate. (−)-Sulpiride (Research Biochemicals Inc.) was dissolved in a minimum volume of concentrated hydrochloric acid, diluted to volume in distilled water and diluted in Krebs buffer. [^{125}I]-angiotensin II (2200 Ci $mmol^{-1}$; NEN) was dissolved in radioligand binding

incubation buffer. All drugs were used as received and were freshly prepared immediately before use.

Statistical analysis

All data are expressed as means ± s.e.. Statistical analysis was performed by one way ANOVA (statistical significance was defined as $P<0.05$) with post hoc tests performed using a Dunnett's t test.

Results

Modulation of dopamine release from rat striatal slices

Basal endogenous dopamine levels varied between experiments (2.14 ± 0.62 pmol min^{-1} mg^{-1} protein, mean ± s.e.mean, $n=27$) and therefore dopamine levels in the perfusates were normalised to basal values to assess the affects of drugs.

Angiotensin II (0.1 – 1.0 μM) induced a concentration-dependent increase in dopamine release from rat striatal slices (Figure 1). Maximal stimulation (approximately 250% above basal levels) was detected within 4 min of agonist application and the response subsequently reduced in the continued presence of angiotensin II (Figure 1). Angiotensin II (1.0 μM) failed to enhance perfusate levels of dopamine in Ca^{2+} free Krebs buffer (data not shown).

Losartan (1.0 μM) failed to modify basal perfusate levels of dopamine but completely antagonized the stimulation induced by angiotensin II (1.0 μM; Figure 1). In contrast, PD123177 (1.0 μM) failed to modify the angiotensin II (1.0 μM)-induced stimulation (Figure 1) and also failed to modify basal perfusate levels of dopamine (Figure 1).

Extracellular striatal levels of dopamine sampled from freely moving rats using the microdialysis technique

The *in vitro* recovery of dopamine using the 'in house' microdialysis probes was approximately 10%. The basal dialysate levels of dopamine varied between experiments (51 ± 12 fmol 20 min^{-1}, means ± s.e.mean, $n=39$) and therefore dopamine levels in the dialysates were normalised to basal values to assess the affects of drugs. The limit of detection for dopamine using the h.p.l.c.–e.c.d. assay was usually between 5–20 fmol on column (signal to noise ratio of 3:1; injection volume 40 μl).

Validation of the neuronal origin of the extracellular dopamine in the striatum

The basal extracellular levels of dopamine in the striatum of freely-moving rats sampled approximately 12 h following the implantation of the microdialysis probe were not modified by the administration of vehicle (Figure 2) but were reduced by over 70% in the presence of the sodium channel blocker, tetrodotoxin (1.0 μM; administered via the microdialysis probe; data not shown).

Modulation of extracellular dopamine levels in the striatum

Angiotensin II (1.0 – 10 μM; administered via the microdialysis probe; probe recovery for angiotensin II was approximately 5% (assessed using [^{3}H]-angiotensin II-spiked angiotensin II, 1.0 μM) induced a concentration-related increase in the extracellular levels of dopamine in the rat striatum (Figure 2). The highest concentration maximally increased dopamine levels by approximately 150% above basal levels (Figure 2); the increase being maximal 20–40 min after the introduction of angiotensin II with the response subsequently declining (Figure 2).

Losartan (1.0 μM; administered via the microdialysis probe) failed to modify extracellular levels of dopamine from the rat striatum and subsequently completely antagonized the elevation of extracellular dopamine levels induced by angiotensin II (10 μM; Figure 2). In the presence of a lower concentration of losartan (0.1 μM), there was a trend for angiotensin II (10 μM) to elevate extracellular dopamine levels although this did not reach statistical significance (Figure 2).

The AT_2 receptor antagonist, PD123177 (1.0 μM; administered via the microdialysis probe) failed to modify either the

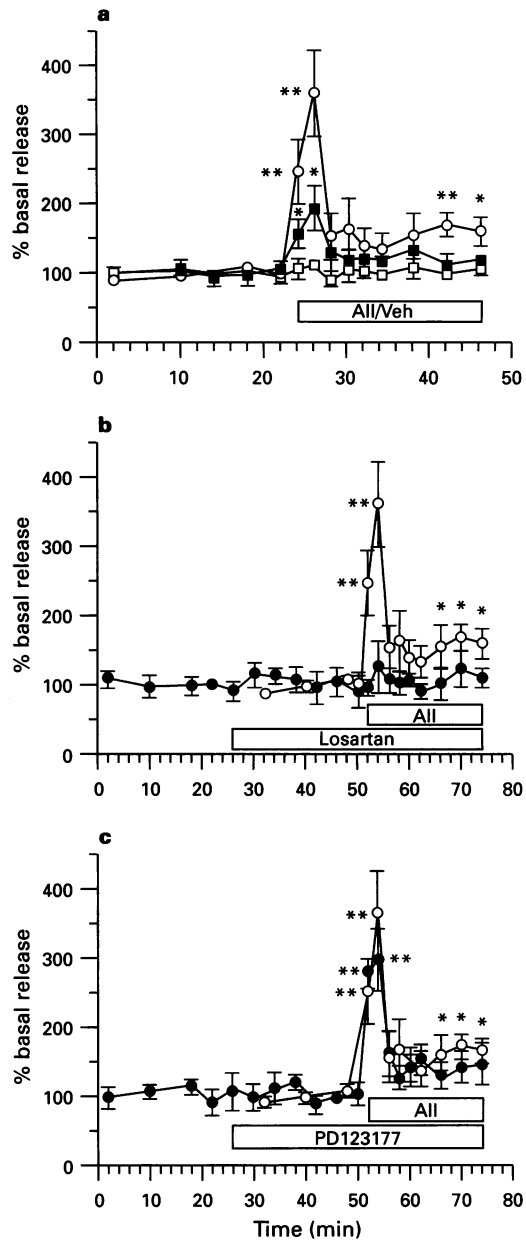

Figure 1 Ability of angiotensin-II (AII; 0.1 – 1.0 μM) to modulate dopamine release from rat striatal slices perfused with Krebs buffer and the interaction with the non-peptide angiotensin-II receptor antagonists, losartan (1.0 μM) and PD123177 (1.0 μM). (a) Angiotensin II (■, 0.1; ○, 1.0 μM) or vehicle (Veh; □); (b) angiotensin-II (○; 1.0 μM) and angiotensin-II (1.0 μM) plus the AT_1 receptor antagonist, losartan (●; 1.0 μM) and (c) angiotensin-II (○; 1.0 μM) and angiotensin-II (1.0 μM) plus the AT_2 receptor antagonist, PD123177 (●; 1.0 μM). Data represent mean ± s.e.mean, $n=4-7$. Horizontal bars represent application of the indicated drug (corrected for the void volume). Comparing dopamine levels in the presence of test compounds with pre-compound basal levels; angiotensin-II (0.1 μM) ANOVA (d.f. 12, 39; $F=2.27$, $P=0.027$); angiotensin-II (1.0 μM) ANOVA (d.f. 12, 65; $F=5.80$, $P<0.001$); angiotensin-II (1.0 μM) plus PD123177 (1.0 μM) ANOVA (d.f. 19, 80; $F=7.09$, $P<0.001$). $^*P<0.05$, $^{**}P<0.01$ (Dunnett's t test).

basal extracellular levels of dopamine or the elevation of extracellular dopamine levels induced by angiotensin II (10 μM; Figure 2).

[125I]-angiotensin II homogenate radioligand binding

[125I]-angiotensin II (0.1 nM) labelled specific binding sites in homogenates of rat parietal cortex, pyriform cortex and striatum (defined by the inclusion of unlabelled angiotensin II, 1.0 μM) which represented approximately 70, 90 and 30–40% of the total binding, respectively. The majority of the specific [125I]-angiotensin II binding in both the pyriform cortex and striatal homogenates was sensitive to losartan (1.0 μM) with a minor portion displaying sensitivity to PD123177 (1.0 μM; Figure 3). In contrast, the majority of specific [125I]-angiotensin II binding in the parietal cortex homogenates was sensitive to PD123177 (1.0 μM) with a more modest component displaying sensitivity to losartan (1.0 μM; Figure 3).

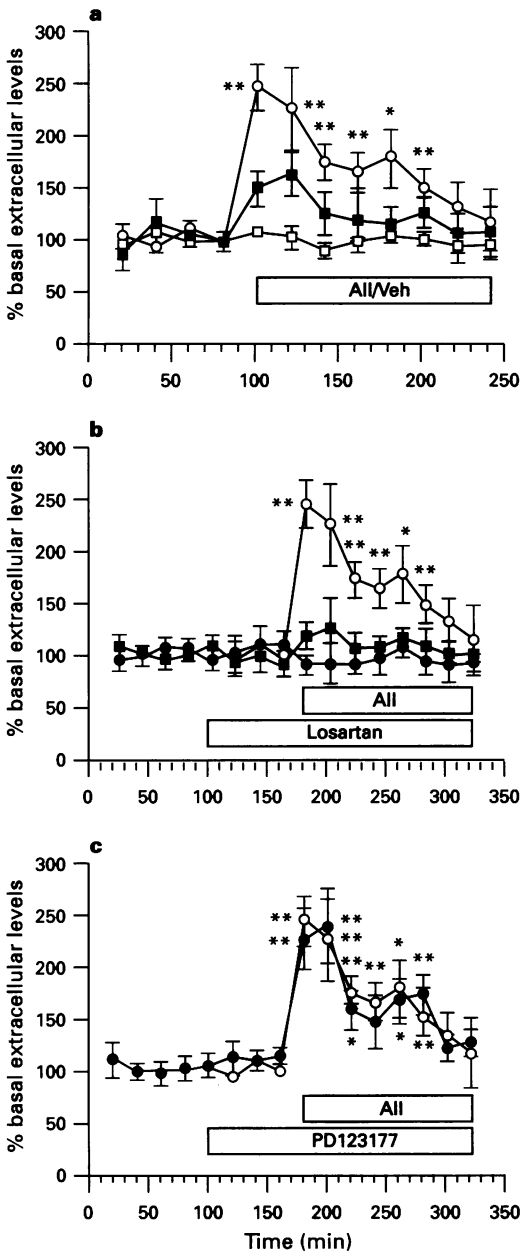

Figure 2 Ability of angiotensin-II (AII; 1.0–10 μM) to modulate extracellular levels of dopamine in the striatum of freely moving rats assessed by the microdialysis technique and the interaction with the non-peptide angiotensin-II receptor antagonists, losartan (1.0 μM) and PD123177 (1.0 μM). (a) Angiotensin II (■, 1.0; ○, 10 μM) or vehicle (Veh; □); (b) angiotensin-II (○; 10 μM) and angiotensin-II (10 μM) plus the AT_1 receptor antagonist, losartan (■, 0.1 μM; ●, 1.0 μM) and (c) angiotensin-II (○; 10 μM) and angiotensin-II (10 μM) plus the AT_2 receptor antagonist, PD123177 (●; 1.0 μM). Data represents mean ± s.e.mean, $n = 5–8$. Horizontal bars represent application of the indicated drug (corrected for the void volume). Comparing extracellular dopamine levels in the presence of test compounds with pre-compound basal levels; angiotensin-II (10 μM) ANOVA (d.f. 11, 84; $F = 5.65$, $P < 0.001$); angiotensin-II (10 μM) plus PD123177 (1.0 μM) ANOVA (d.f. 15, 96; $F = 4.83$, $P < 0.001$). $^*P < 0.05$, $^{**}P < 0.01$ (Dunnett's t test).

Discussion

In the present studies, an *in vitro* and an *in vivo* neurotransmitter release preparation was used to demonstrate that angiotensin II, via the AT_1 receptor, modulates striatal dopamine release. Both preparations displayed the expected physiological characteristics to indicate that the quantified dopamine was neuronal in origin. Thus using the *in vitro* preparation, we have demonstrated that the dopamine release is potentiated by potassium ions in a Ca^{2+}-dependent manner (Steward *et al.*, 1996). Furthermore, in the present series of experiments, the stimulation of dopamine release from striatal slices induced by angiotensin II was prevented when Ca^{2+} was omitted from the perfusing Krebs buffer (data not shown). Similarly, the ability of the sodium channel blocker, tetrodotoxin, to reduce dramatically extracellular levels of striatal dopamine sampled using the *in vivo* microdialysis technique indicates that at least the majority of the quantified dopamine was neuronal in origin.

In both the *in vitro* striatal slice and the *in vivo* striatal microdialysis preparations, angiotensin II elevated dopamine release in a concentration-related manner. Effective concentrations of angiotensin II in both preparations correlated when a 'recovery' factor was taken into account (estimated to be approximately 5% due to the permeable barrier imposed by the microdialysis membrane) and were comparable to angiotensin II concentrations which modulate neurotransmitter release in other preparations (e.g. Diz & Pirro, 1992; Brasch *et al.*, 1993).

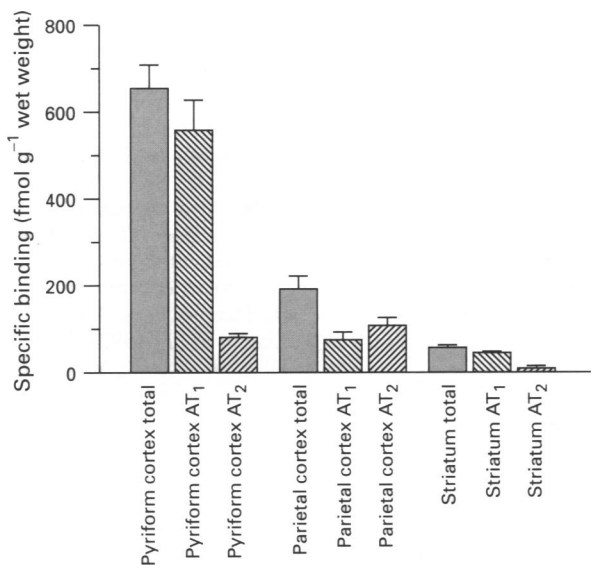

Figure 3 Levels of [125I]-angiotensin-II (0.1 nM) specific binding to homogenates of rat pyriform cortex, parietal cortex and striatum. Total specific binding was defined by the presence of unlabelled angiotensin-II (1.0 μM) whilst AT_1 and AT_2 receptor specific binding levels were defined by the presence of losartan (1.0 μM) and PD123177 (1.0 μM), respectively. Data represent the mean ± s.e.mean, $n = 3$.

The maximal increase in dopamine release was approximately 250% and 150% above basal levels for the *in vitro* and *in vivo* preparations, respectively. Although higher concentrations of angiotensin II than those evoking these levels of release were not evaluated in the present studies, assuming a normal concentration-response relationship, it is unlikely that the maximal increases attainable induced by angiotensin II would be considerably higher since a 10 fold lower concentration of angiotensin II in both preparations evoked smaller, but still statistically significant responses (approximately 40% of the response evoked by the 10 fold higher concentration of angiotensin II).

After the demonstration that angiotensin II enhanced striatal dopamine release, subsequent experiments were performed with both the *in vitro* and *in vivo* release preparations to characterize pharmacologically the receptor mediating the response. For this purpose the non-peptide AT receptor antagonists, losartan (formerly designated DuP753) and PD1232177 were used. These structurally unrelated compounds display around 3 orders of magnitude selectivity and display nanomolar affinity for the AT_1 and AT_2 receptor, respectively (for review see Chiu *et al.*, 1994). Hence, in the present studies the ability of losartan, and the failure of PD123177, to antagonize completely the angiotensin II-induced stimulation of dopamine release in both the *in vitro* striatal slice preparation and the *in vivo* microdialysis preparation provides strong evidence that the response was mediated via the AT_1 receptor. At least in the rat, the AT_1 receptor can be further subdivided into two subtypes encoded by separate genes, namely AT_{1A} and AT_{1B} receptors (Iwai *et al.*, 1991; Murphy *et al.*, 1991; Kakar *et al.*, 1992b). The mRNAs encoding these receptors are differentially distributed in the brain (Bunnemann *et al.*, 1992; Kakar *et al.*, 1992a), although to date, no report has identified the mRNA encoding either receptor subtype in the substantia nigra (containing the cell bodies of the dopaminergic neurones of the nigro-striatal pathway). Unfortunately, no pharmacological compound has been reported to discriminate between these two receptors (e.g. Balmforth *et al.*, 1994) and therefore further subdivision of the AT_1 receptor mediating dopamine release was not attempted.

Our findings are consistent with previous reports indicating that striatal dopamine release is stimulated via angiotensin II. These studies, however, were either performed prior to the availability of AT receptor subtype selective compounds (preventing the classification of the AT receptor responsible for the response; Simonnet & Giorguieff-Chesselet, 1979) or the characterization of the angiotensin II-induced response was evaluated by a more indirect method to assess dopamine release (e.g. modulation of the extracellular levels of dopamine metabolites or whole tissue levels of dopamine and its metabolites; Braszko *et al.*, 1992; Dwoskin *et al.*, 1992; Mendelsohn *et al.*, 1993).

It was of interest that the stimulation of dopamine release induced by angiotensin II in both preparations declined in the continued presence of angiotensin II, indicative of the receptor being prone to desensitization as has been reported previously for responses mediated via the AT_1 receptor (e.g. Abdellatif *et al.*, 1991; Kuttan & Sim, 1993; Reagan *et al.*, 1993). It should be noted, however, that the time course of the apparent desensitization of the response was markedly different between the two preparations and it could not be ruled out that at least a component of the apprent desensitization was due to a depletion of neuronal dopamine levels.

Given that the responses elicited by angiotensin II in the present studies were evoked by application of the agonist to either striatal slices or the local application to the striatum in the intact brain, the AT_1 receptors mediating the responses are presumably located within the striatum. It is therefore intriguing that radioligand binding studies have consistently demonstrated either relatively very low levels of AT receptors in the adult rat striatum (Sirett *et al.*, 1977; Mendelsohn *et al.*, 1984) or levels often below the limits of detection (e.g. see Gehlert *et al.*, 1991). Since it has been demonstrated that clear species differences exist with respect to the relative level of AT receptor density within the striatum (with relatively high levels of AT receptors in the human striatum; Allen *et al.*, 1992; Barnes *et al.*, 1993; MacGregor *et al.*, 1995) and also that AT receptor densities display marked ontological variations (with relatively high levels during foetal and neonatal development; e.g. Millan *et al.*, 1991; for review see Bottari *et al.*, 1993), in the present study the levels of AT receptors in the striatum of rats of the same strain and age as were used for the release experiments were assessed. Whilst we were unable to detect reproducibly [^{125}I]-angiotensin II specific binding in the striatum of sections of rat brain using quantitative receptor autoradiography (although relatively high levels were detectable in other brain regions, e.g. pyriform cortex, cerebellum, dorsal vagal complex; Steward and Barnes, unpublished observations), [^{125}I]-angiotensin II-labelled AT receptors were detected in striatal membranes. This presumably reflects a higher level of sensitivity for detecting membrane bound receptors in this latter preparation. In addition, the majority of the specific [^{125}I]-angiotensin II binding in the striatal membranes appeared to be associated with the AT_1 receptor since it was sensitive to the selective AT_1 receptor antagonist, losartan, but insensitive to the selective AT_2 receptor antagonist, PD123177. These sites may be responsible for the angiotensin II-induced modulation of dopamine release demonstrated in the present study. Although the absolute number of receptors required to evoke responses in a given tissue may be debated, the cellular location and density of the receptor is probably of greater importance. This information is not available for the AT_1 receptor in the rat striatum, but it is of interest that in the human brain, a population of AT_1 receptors appear to be associated with the dopamine neurones of the nigro-striatal pathway (Allen *et al.*, 1992; Barnes *et al.*, 1993).

In summary, the present studies provide direct evidence that dopamine release in the rat striatum is modulated by the AT_1 receptor.

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