Pharmacological characterization of the dopamine receptor coupled to cyclic AMP formation expressed by rat mesenteric artery vascular smooth muscle cells in culture

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1 Mesenteric artery vascular smooth muscle cells derived from male Wistar rats and grown in culture were prelabelled with [³H]-adenine and exposed to a range of dopamine receptor agonists and antagonists. Resultant [³H]-cyclic AMP formation was determined and concentration-effect curves constructed, in the presence of propranolol (10^{-6} M) and the phosphodiesterase inhibitor IBMX (5×10^{-4} M).

2 K_a apparent values for D_1/DA_1 dopamine receptor agonists SKF 38393, fenoldopam, 6,7-ADTN, and dopamine were 0.06, 0.59, 4.06 and 5.77×10^{-6} M respectively. Although fenoldopam and SKF 38393 were more potent than dopamine, they were partial agonists with efficacies, relative to dopamine of approximately 48% and 24% respectively. 6,7-ADTN, in contrast, behaved as a full agonist.

3 Dopamine-stimulated cyclic AMP formation was inhibited in a concentration-dependent manner by the D_1/DA_1 dopamine receptor selective antagonists, SCH 23390 and *cis*-flupenthixol (K_i values 0.53 and 36.1×10^{-1} M respectively). In contrast, the D_2/DA_2 dopamine receptor selective antagonists, domperidone and (–)-sulpiride, were less potent (K_i values 2.06 and 5.82×10^{-6} M respectively). Furthermore, the stereoisomers of SCH 23390 and *cis*-flupenthixol, SCH 23388 and trans-flupenthixol, were at least two orders of magnitude less potent (K_i values 0.14 and 13.2×10^{-6} M respectively) indicating the stereoselective nature of this receptor.

4 Our results indicate that rat mesenteric artery vascular smooth muscle cells in culture express a dopamine receptor coupled to cyclic AMP formation, which has the pharmacological profile, characteristic of the D_1 dopamine receptor subfamily.

Keywords: Rat mesenteric artery; cyclic AMP; dopamine receptor; vascular smooth muscle

Introduction

Specific dopamine receptors located on cells within the renal vasculature and cortex are now known to be responsible for inducing vasodilatation, diuresis and natriuresis by linkage to the enzymes adenylate cyclase and phospholipase C (Anderson *et al.*, 1990). These receptors have been further localized to the vascular media and renal tubules, whilst a second pharmacologically distinct receptor subtype, has been identified on the endothelial and neuronal cells of the vascular intima and adventitia (Amenta, 1990).

Cardiovascular dopamine receptors have traditionally been referred to using the DA_1/DA_2 dopamine receptor nomenclature proposed by Goldberg & Kohli in 1979. This scheme contrasts with one suggested by Keabian & Calne earlier that same year (Keabian & Calne, 1979). These authors proposed a D_1/D_2 nomenclature for dopamine receptors whether located within the periphery or within the central nervous system. The use of separate nomenclatures, whilst based primarily on anatomical location, has persisted due to some reported differences in pharmacological profiles, particularly with regard to the relative potency of partial agonists SKF 38393 and fenoldopam (SKF 82526). However, it remains unclear the extent to which these anomalies result from the differing experimental techniques used in the study of dopamine receptors at each location rather than true differences in receptor pharmacology at these sites (Hieble, 1987).

We have developed an intact cell culture model of the peripherally derived, adenosine 3':5'-cyclic monophosphate (cyclic AMP) stimulating, vascular DA_1 dopamine receptor. This approach is identical to the one previously used by

members of our group to study the centrally derived D_1 dopamine receptor (Balmforth *et al.*, 1988b) and also the peripherally derived DA_1 dopamine receptor (Bryson *et al.*, 1992) expressed by human glial, and rat glomerular mesangial cells respectively. Vascular smooth muscle cell cultures derived from the rat superior mesenteric arterial-tree represent the small calibre resistance vessels that are of importance in the regulation of blood pressure and also regional blood flow (Campbell & Campbell, 1987). We have reported preliminary evidence that these cells in culture express a dopamine receptor coupled to the stimulation of cyclic AMP (Balmforth *et al.*, 1988a) and now describe a detailed pharmacological profile of the dopamine receptor expressed in this system.

Methods

Cell culture

Rat mesenteric artery vascular smooth muscle cells were cultured by a modification of the technique of enzymatic dissociation (Gunther *et al.*, 1982). Briefly, four male Wistar rats weighing 150–250 g were killed by stunning, cervical dislocation and decapitation. Following excision of the mesenteric vascular tree under sterile conditions, the vessels were cleaned of excess adipose and adventitial tissue and the mesenteric veins discarded. Arteries were then cut into 1 mm segments and placed in 5 ml of enzyme dissociation mixture (serum-free medium containing; collagenase 2.5 mg ml⁻¹, elastase 0.05 mg ml⁻¹ and soyabean trypsin inhibitor 0.5 mg ml⁻¹). Following 10 min incubation at 37°C, any residual adipose, adventitial, or endothelial tissues were

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removed by a process of trituration and centrifugation (1 min, 600 g). Muscular tubes derived in this way were placed in 10 ml of fresh enzyme dissociation mixture and incubated at 37°C for a further 30 min, being triturated periodically to facilitate the process of tissue dissociation. Cells were harvested by centrifugation at 600 g for 6 min, and suspended in 5 ml of Dulbecco's modified Eagle's medium (DMEM) containing 20% foetal calf serum, 2×10^{-6} M L-glutamine, 1,000 u ml⁻¹ penicillin G sodium, 100 µg ml⁻¹ streptomycin sulphate, 0.25 µg ml⁻¹ amphotericin B and 100 µg ml⁻¹ gentamycin. Confirmation of the purity and identity of cells cultured was obtained by phase microscopy and by staining cells with a fluorescent antibody to rat vascular smooth muscle α -actin. Cells were seeded into a 25 cm² tissue culture flask and maintained in a humidified atmosphere of 2% CO₂, 98% air at 37°C with medium being changed at intervals of 2–3 days. Cells for assays were subcultured in 6-well multidishes each containing 4 ml of medium and plated at a seeding density of 2×10^4 cells ml⁻¹. Assays were performed 7 days after subculture, using cells between the second and fifth passage in all experiments.

Assay for cyclic AMP formation in intact cells

The method for measurement of intracellular cyclic AMP formation is a modification of the prelabelling technique as described previously by (Balmforth *et al.*, 1988b). Briefly, cell adenine nucleotide pools were labelled by replacing used with fresh medium (2 ml), containing 4 µCi of [³H]-adenine, and incubating at 37°C for 2 h, after which cells were washed three times with serum-free medium (2 ml). Cyclic AMP formation was stimulated by addition of agonists (when appropriate in the presence of antagonists) in serum-free medium containing the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX, 0.5 mM). Propranolol (10^{-6} M) was present for all experiments except for those in which dopamine agonist activity at α -, β -adrenoceptor, and DA₂ dopamine receptor sites was investigated. This concentration of propranolol was chosen as it was observed to inhibit isoprenaline (10^{-5} M) without attenuating cyclic AMP formation stimulated by D₁/DA₁ dopamine receptor agonists SKF 38393 (10^{-5} M) and fenoldopam (10^{-5} M). All antagonist concentration-effect studies assessed inhibition of dopamine-induced (10^{-4} M) cyclic AMP formation. After 12 min at 37°C (time of maximal cyclic AMP formation) incubations were terminated by aspiration of the medium and subsequent addition of 1.5 ml of ice-cold 5% trichloroacetic acid (TCA) containing [¹⁴C]-cyclic AMP (1.25 nCi ml⁻¹) as an internal standard. Cyclic AMP was isolated from the TCA extracts by sequential chromatography on Dowex AG50W-X4 anion exchange and neutral alumina columns (Salomon *et al.*, 1974). The [³H]-cyclic AMP content of each sample was corrected for quench and [¹⁴C]-cyclic AMP recovery (60–80%) and expressed in units of d.p.m. well (disintegrations per minute per well) using a Hewlett Packard 2000CA scintillation counter.

Data analysis

Concentration-effect curves were drawn for agonists and antagonists using the 'Graphpad' iterative curve-fitting computer programme (ISI Software). Sigmoid curves generated from the experimental data points ($\pm$ s.e. mean) were not constrained by fixing any numeric parameters such as the slope, or the maximum, minimum and EC₅₀/IC₅₀ values. In this way the K_a apparent affinity constant was determined objectively for each agonist studied. A K_i value was determined using the Cheng-Prusoff equation, $K_i = IC_{50}/1 + (L/K_a)$ where IC₅₀ = concentration of antagonist required to inhibit 50% of dopamine-stimulated responses, L = concentration of dopamine used in the assay and K_a = concentration of dopamine producing 50% of the maximal dopamine-stimulated cyclic AMP response (Cheng & Prusoff, 1973). All

results are expressed as the geometric mean with the upper and lower 95% confidence limits in parentheses.

Materials

Dopamine hydrochloride, ($\pm$)-propranolol hydrochloride, 3-isobutyl-1-methylxanthine (IBMX), collagenase (type 1), elastase (type 1), and soyabean trypsin inhibitor were purchased from Sigma Chemicals (Poole, Dorset.) SCH 23388 (S-[7-chloro-8-hydroxy-3-methyl-1-phenyl-2, 3, 4, 5-tetrahydro-1H-3-benzazepine hydrochloride), (+)-SKF 38393 (R-[+]-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,8-diol hydrochloride), 6,7-ADTN (2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene hydrochloride) and (–)-sulpiride from Semat (U.K.) Ltd. (St. Albans, Hertfordshire); [³H]-adenine and adenine [U-¹⁴C]-cyclic AMP from Amersham International P.L.C. (Amersham, Bucks). Dowex AG50W-X4 (200–400 mesh) and neutral alumina AG7 (100–200 mesh) from Biorad (Watford, Hertfordshire). All tissue culture reagents and plastics were purchased from Gibco (Paisley, Scotland). The following reagents were generously donated: SCH 23390 (R-[+]-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine-maleate) from Schering (Bloomfield, NJ, U.S.A.); *cis*-flupenthixol and *trans*-flupenthixol from H. Lundbeck A/S (Copenhagen, Denmark); domperidone maleate from Janssen Pharmaceuticals (Wantage, Oxon); fenoldopam methane sulphate from Smith, Kline and French Research (Welwyn, Hertfordshire).

Results

Effect of catecholamine receptor antagonists

Addition of dopamine (10^{-4} M) to vascular smooth muscle cells produced an approximate six-fold increase in cyclic AMP formation as compared to the control values obtained for cells exposed to IBMX (5×10^{-4} M) alone. This stimulation was equivalent to that observed for isoprenaline (10^{-5} M) though only a fifth of that observed for forskolin (10^{-5} M) (data not shown). Dopamine (10^{-4} M)-stimulated cyclic AMP formation was not potentiated by the addition of the α -adrenoceptor antagonist phentolamine (10^{-6} M) nor the D₂/DA₂ dopamine receptor antagonist (–)-sulpiride (10^{-6} M) (Figure 1). The β -adrenoceptor antagonist, propranolol (10^{-6} M) produced either partial or no inhibition of dopamine-induced cyclic AMP formation. Furthermore the increase in cyclic AMP levels produced by dopamine was almost completely inhibited by the D₁/DA₁-dopamine receptor antagonist, SCH 23390 (10^{-6} M). These initial data suggested the presence of both DA₁-dopamine and β -adrenoceptors linked to cyclic AMP formation. As a small effect of dopamine mediated via β -adrenoceptors could not be excluded, and to prevent any confounding effects of the other dopamine receptor agonists via the β -adrenoceptors expressed in this system (Hall *et al.*, 1992) further characterization of the dopamine receptor was undertaken in the presence of propranolol (10^{-6} M).

Effect of dopamine receptor agonists

Each agonist studied produced a concentration-related stimulation of cyclic AMP formation, though the benzazepines, SKF 38393 and fenoldopam (SKF 82526), were observed to be partial agonists with efficacies (maximal levels of stimulated cyclic AMP formation) relative to dopamine (10^{-4} M) of approximately 24% and 48% respectively (Figure 2). When the relative potencies of the different dopamine receptor agonists investigated were compared on the basis of their K_a apparent values (agonist concentration giving half-maximal stimulation) the following potency series was observed; SKF 38393 > fenoldopam > 6, 7-ADTN = dopamine, with K_a apparent values of 0.06 (0.02–0.07), 0.59

Figure 1 The effects of 10^{-6} M phenolamine, propranolol, SCH 23390, and $(-)$ -sulpiride (acting at α , β adrenoreceptors, D_1/DA_1 and D_2/DA_2 dopamine receptors, respectively) on dopamine-induced (10^{-4} M) cyclic AMP formation. Each column is the mean ($\pm$ s.e.mean) of triplicate determinations of a single representative experiment.

Figure 2 The stimulation of cyclic AMP formation induced by D_1/DA_1 dopamine receptor agonists $(+)$ -SKF 38393 ($\bullet$), fenoldopam ($\circ$), 6,7-ADTN ($\blacksquare$) and dopamine ($\square$). K_A apparent values were 0.06, 0.59, 4.06, and 5.77×10^{-6} M respectively. Dopamine (10^{-4} M) was included as a standard with each concentration-effect curve, in order to determine the efficacy of each drug. Each point is the mean (s.e.mean) of at least three separate experiments expressed as a percentage of maximal cyclic AMP formation induced by dopamine (13.40, 5.54, 4.78 and 12.94 thousands d.p.m./well respectively) after basal values have been subtracted (1.51, 1.15, 1.34 and 2.00 thousands d.p.m./well respectively).

(0.59–0.60), 4.06 (1.50–5.68), and $5.77 (4.04–7.26) \times 10^{-6}$ M, respectively.

Effect of dopamine receptor antagonists

The D_1/DA_1 dopamine receptor antagonists, SCH 23390 (10^{-6} M) and *cis*-flupenthixol (10^{-4} M), completely inhibited maximal dopamine-induced (10^{-4} M) cyclic AMP production, achieving 50% inhibition in the sub-micromolar range (Figure 3). In contrast, the dopamine D_2/DA_2 receptor antagonists domperidone and $(-)$ -sulpiride were observed to be significantly less potent inhibitors of dopamine-induced (10^{-4} M) cyclic AMP formation. K_i values were 0.53 (0.27–1.15), 36.1 (27.55–90–84), 2,060 (1573–3,493) and $5,820 (4,015–7,332) \times 10^{-9}$ M respectively. The potency series observed for these antagonists was therefore SCH 23390 > *cis*-flupenthixol >> domperidone = $(-)$ -sulpiride. The potencies of dopamine D_1/DA_1 receptor antagonists SCH 23390 and *cis*-flupenthixol were also compared with the potencies of their stereo-isomers SCH 23388 and *trans*-flupenthixol, in

Figure 3 Concentration-effect curves for the D_1/DA_1 dopamine selective antagonists, SCH 23390 ($\bullet$) and *cis*-flupenthixol ($\square$) as compared to D_2/DA_2 dopamine selective antagonists domperidone ($\blacksquare$) and $(-)$ -sulpiride ($\circ$). K_i values were 0.53, 36.1, 2,060, and $5,820 \times 10^{-9}$ M respectively. Each point is the mean ($\pm$ s.e.mean) of at least three separate experiments expressed as a percentage of dopamine-induced (10^{-4} M) cyclic AMP formation in the absence of any antagonist (8.76, 4.53, 6.43 and 5.01 thousands d.p.m./well respectively) and with basal values subtracted (2.49, 1.47, 1.44 and 2.08 thousands d.p.m./well respectively).

Figure 4 The relative potency of D_1/DA_1 dopamine receptor antagonists SCH 23390 ($\circ$) and *cis*-flupenthixol ($\square$) as compared with their stereo-isomers SCH 23388 ($\bullet$) and *trans*-flupenthixol (Δ). K_i values were 0.53, 36.1, 143, and $13,240 \times 10^{-9}$ M respectively. Each point is the mean ($\pm$ s.e.mean) of at least three separate experiments expressed as a percentage of dopamine-induced (10^{-4} M) cyclic AMP formation in the absence of any antagonist (8.76, 4.53, 5.42 and 1.14 thousands d.p.m./well respectively) and with basal values subtracted (2.49, 1.47, 1.51 and 1.14 thousands d.p.m./well respectively).

order to investigate the stereo selective properties of the receptor (Figure 4). Both SCH 23388 and *trans*-flupenthixol were at least two orders of magnitude less potent than their stereo-isomers, with K_i values of 143(53–149) and 13,240 (3,200–22,580) nM respectively.

Discussion

We have confirmed our previous finding (Balmforth *et al.*, 1988a) that rat mesenteric artery vascular smooth muscle cells in culture express dopamine receptors linked to the stimulation of adenylate cyclase. Moreover, by using intact cells, instead of homogenates as previous workers (Murthy *et al.*, 1976; Nakajima *et al.*, 1977; Kotake *et al.*, 1981) an

increased contrast, between basal and maximal stimulation of cyclic AMP by dopamine, has been achieved. This enhanced resolution is comparable to that observed for intact-cell models of the dopamine receptors located on bovine parathyroid (Brown *et al.*, 1980), human glial (Balmforth *et al.*, 1988b) and also rat mesangial cells (Bryson *et al.*, 1992). Increased sensitivity, combined with the use of selective dopamine receptor agonists and antagonists, allowed us to characterize more fully the vascular dopamine receptor. Furthermore, the use of identical experimental techniques to those previously used by our group to characterize dopamine receptors expressed by mesangial and glial cell types (Balmforth *et al.*, 1988b; Bryson *et al.*, 1992) permits direct comparison of observed pharmacological profiles.

Several other properties make this experimental model particularly suitable for studying the pharmacological and biochemical properties of the vascular dopamine receptor (Furchgott, 1972). These features include (a) the virtual absence of contamination with other vascular and non-vascular cell types (b) the ability to manipulate the extracellular environment and maintain controlled experimental conditions for relatively long periods and (c) the ability to study receptor-coupled events without the artifact of tissue diffusion barriers and without the disruption of cell membranes and of subcellular structures.

The benzazepine compound SCH 23390 is a potent and selective D_1/DA_1 dopamine receptor antagonist (Hyttel, 1983) with a K_i in the nanomolar range. We observed concentration-dependent inhibition of dopamine-induced stimulation of cyclic AMP formation with this compound (K_i 0.53×10^{-9} M). By comparison, the weakly active stereoisomer of SCH 23390, SCH 23388, was a poor antagonist of dopamine-induced increases in cyclic AMP. Thus as observed initially for the D_1 dopamine receptor expressed by rat striatal homogenates (Stoof & Keabian, 1984; Barnett *et al.*, 1986) and subsequently for the D_1 dopamine and DA_1 dopamine receptors expressed by human glial and rat mesangial cells in culture (Balmforth *et al.*, 1988b; Bryson *et al.*, 1992) the orientation of the phenyl substituent of SCH 23390 is critical for potent antagonism. In addition, stereoselectivity was also apparent from the difference (greater than two orders of magnitude) in the concentration-effect curves of the stereoisomers *cis*- and *trans*-flupenthixol.

In contrast to both SCH 23390 and *cis*-flupenthixol the D_2/DA_2 dopamine receptor selective antagonists domperidone and (-)-sulpiride were observed to be of very low potency. Furthermore, no enhancement of dopamine-induced stimulation of cyclic AMP formation was observed in the presence of either domperidone or (-)-sulpiride, indicating the absence of DA_2 dopamine receptors, coupled to the inhibition of adenylate cyclase, in this preparation. Some previous investigators have suggested that vascular tissues may express DA_2 dopamine receptors (Missale *et al.*, 1988; Munch *et al.*, 1991). However, it should be noted that both groups performed radio ligand binding studies on membrane preparations derived from homogenized arteries. It is highly probable, therefore, that neuronal and endothelial cell types, possibly expressing the DA_2 dopamine receptor subtypes, were also present. Detailed autoradiographic imaging indicates that dopamine DA_2 receptors are likely to be located within the adventitia and endothelium, having no direct association with vascular smooth muscle cells (Amenta, 1990). Our own data would strongly support these findings, indicating localization of a DA_1 dopamine but not a DA_2 dopamine receptor on vascular smooth muscle cells.

Vascular smooth muscle cells derived from the rat mesenteric arterial-tree also express β -adrenoceptors both *in vivo* (Nichols & Hiley, 1985) and *in vitro* (Hall *et al.*, 1992). For this reason all dopamine receptor characterization studies were performed in the presence of the β -adrenoceptor antagonist propranolol (10^{-6} M). In contrast with *in vivo* observations, isolated vascular smooth muscle cells in culture do not express functional α -adrenoceptors (Bobik, 1987). No

adequate explanation for this interesting observation has yet been proposed. However, irrespective of the mechanism, the absence of α -adrenoceptors in our system conveniently avoids the need for an α -adrenoceptor antagonist to prevent dopamine interactions at these sites.

We have observed that 6,7-ADTN is a full agonist at the vascular dopamine receptor, equipotent to dopamine, and with a K_a apparent value in the micromolar range. This is consistent with previous reports for dopamine receptors, coupled to adenylate cyclase, derived both from the periphery (Kotake *et al.*, 1981; Bryson *et al.*, 1992) and also those derived from the central nervous system (Balmforth *et al.*, 1988b). The vascular dopamine receptor expressed in our experimental system demonstrated a greater affinity for D_1/DA_1 dopamine receptor selective agonists, SKF 38393 and fenoldopam, than for either 6,7-ADTN or dopamine, giving the following relative potency series, SKF 38393 > fenoldopam > 6,7-ADTN = dopamine. However, both SKF 38393 and fenoldopam stimulated much lower maximal levels of cyclic AMP formation than either dopamine or 6,7-ADTN giving the relative efficacy series 6,7-ADTN = dopamine > fenoldopam > SKF 38393. Our potency series is consistent with those reported for radioligand binding and second messenger studies of the centrally derived D_1 dopamine (Balmforth *et al.*, 1988b; Anderson *et al.*, 1990; Sibley & Monsma, 1992), and also the peripherally derived DA_1 dopamine receptors (Hughes & Sever, 1989; Bryson *et al.*, 1992). However, these contrast with the potency series reported for the DA_1 dopamine receptor when studied using the physiological endpoint of vasorelaxation (Cavero *et al.*, 1982; Hilditch & Drew, 1985; Anderson *et al.*, 1990). Though at first this would appear to represent a discrepancy, further consideration suggests that it is not. Both SKF 38393 and fenoldopam act as partial agonists at the vascular DA_1 dopamine receptor in this, as in most other *in vitro* experimental systems. For example Hilditch & Drew (1985) report that SKF 38393 produced no detectable relaxation of isolated rabbit splenic arteries. However, by using SKF 38393 as an antagonist instead, they were able to confirm the DA_1 dopamine receptor of the high affinity for this compound.

A further explanation of the discrepancy between our own, and previous reported agonist potency series for the DA_1 dopamine receptor, concerns the contrasting use of the α -adrenoceptor antagonist phenoxybenzamine. Many *in vitro* and *in vivo* experiments have been performed following exposure to large concentrations of phenoxybenzamine (3×10^{-5} M for up to 60 min; Goldberg & Kohli, 1979; Brodde, 1982). This compound has often been used in preference to alternative more selective α -adrenoceptor antagonists as it also inhibits reuptake of noradrenaline into sympathetic nerve terminals and thereby prevents the confounding effects of endogenous catecholamine release (Cuceddu *et al.*, 1974). However, we have observed that phenoxybenzamine produces potent and specific inhibition of the vascular dopamine receptor (Hall *et al.*, 1993). Possible alkylation of DA_1 dopamine receptor sites by phenoxybenzamine, would be expected to result in a reduction of receptor number. Because the maximum response produced by a partial agonist requires occupation of all expressed receptors, the effects of fenoldopam and SKF 38393 may be markedly attenuated as a result. In contrast the effect produced by the full agonists dopamine and 6,7-ADTN might be expected to remain relatively intact, if spare receptors were present.

The potency series for both antagonists and agonists which we have observed in this series of investigations, are consistent with those described for the centrally derived human glial cell D_1 dopamine receptor, and the peripherally derived rat glomerular mesangial DA_1 dopamine receptor (Balmforth *et al.*, 1988b; Bryson *et al.*, 1992) previously characterized by members of our group using identical experimental techniques. Furthermore, our results are consistent with radioligand binding studies performed on membrane preparations derived from homogenates of the rat striatum (Stoof &

Kebabian, 1984) and also from the human renal cortex (Hughes & Sever, 1989). This leads us to the conclusion that rat mesenteric artery vascular smooth muscle cells in culture express a dopamine receptor coupled to cyclic AMP formation, which has the pharmacological profile, characteristic of the D₁ dopamine receptor subfamily (Sibley & Monsma, 1992).

Attempts to identify mRNA of the recently sequenced, centrally derived, dopamine D₁ and D₂ receptor subtypes, in homogenized tissues derived from the rat liver, heart, kidney, lung, and spleen, have so far been unsuccessful (Dearry *et al.*, 1990; Monsma *et al.*, 1990; Sunahara *et al.*, 1990; 1991). It remains unclear however, whether this failure has been due to a lack of resolution in the techniques which have been employed in these studies, or whether it has been due to the

absence of the D₁ and D₂ dopamine receptor subtypes within the periphery. Such data suggest, that despite identical pharmacological profiles, the peripherally derived vascular dopamine receptor may be structurally distinct from cyclic AMP, stimulating dopamine receptors expressed within the central nervous system. The continued though perhaps temporary, use of the DA₁ dopamine receptor nomenclature therefore provides a helpful distinction when describing the vascular dopamine receptor. However, were future work to confirm that the DA₁ dopamine receptor is structurally distinct from both the D₁ (D_{1A}) and the D₂ (D_{1B}) subtypes, then adoption of the D_{1C} receptor nomenclature proposed by Sibley & Monsma (1992) would become much more appropriate.

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