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Pharmacological evidence for the presence of a peripheral postjunctional D_2-like dopamine receptor in rabbit splenic artery

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1 This study was designed to investigate the involvement of postjunctional D_2-like receptors in a rabbit vasculature model used to evaluate the D_1-like agonist activity. Dopamine, epinine and (−)-DP-5,6-ADTN, three mixed D_1/D_2-like agonists, fenoldopam and SKF 82958, two selective D_1-like agonists and SKF 89124, a selective D_2-like agonist, were administered cumulatively in precontracted and α/β-blocked rabbit splenic artery rings in order to evaluate their D_1-like-mediated vasorelaxant activity before and after pretreatment with the selective D_2-like antagonist YM 09151-2 (1 nM).

2 Dopamine ($pD_2 = 6.35 \pm 0.09$), epinine ($pD_2 = 6.73 \pm 0.13$), (−)-DP-5,6-ADTN ($pD_2 = 7.56 \pm 0.09$) and SKF 82958 ($pD_2 = 8.55 \pm 0.10$) reversed completely the U46619-induced contracture whereas SKF 89124 was inactive up to 10 μM and fenoldopam acted like a partial agonist ($pD_2 = 8.31 \pm 0.09$, $\alpha = 0.62$). The selective D_2-like dopamine receptor antagonist YM 09151-2 (1 nM) significantly ($P < 0.05$) potentiated the vasorelaxant activity of dopamine ($pD_2 = 7.01 \pm 0.07$), epinine ($pD_2 = 7.14 \pm 0.08$), (−)-DP-5,6-ADTN ($pD_2 = 8.19 \pm 0.09$) and SKF 89124 (40% relaxation at 10 μM), whereas it did not alter the effects of fenoldopam ($pD_2 = 8.40 \pm 0.09$, $\alpha = 0.68$) and SKF 82958 ($pD_2 = 8.58 \pm 0.08$).

3 The D_2-like antagonist YM 09151-2 induced the same degree of effect with all the substances tested in both endothelium-denuded and endothelium-intact preparations.

4 The selective D_2-like dopamine receptor agonist SKF 89124 did not produce any intrinsic effect on the splenic artery, but was able to produce a rightward shift of the forskolin-induced relaxation.

5 The results of these experiments support the existence of a non-endothelial postjunctional D_2-like dopamine receptor counteracting the D_1-like-mediated vasodilatation in rabbit splenic artery, probably by the inhibition of adenylate cyclase.

Keywords: Peripheral dopamine receptors; postjunctional D_2-like receptors; rabbit isolated splenic artery; dopamine agonists

Introduction

Peripheral dopamine receptors have been identified in vascular smooth muscle and on the terminals of postganglionic sympathetic nerves and have been classified as D_1 and D_2 receptors, respectively (Goldberg & Kohli, 1983; Hilditch & Drew, 1985). Due to the variety of dopamine receptors recently discovered, we will use the term D_1-like for the D_1 and D_5 receptors and D_2-like for the D_2, D_3 and D_4 receptors (Sibley & Monsma, 1992). At the vascular level the activation of D_1-like receptors mediates directly relaxation in different vascular beds through activation of adenylate cyclase (AC) and subsequent generation of the intracellular second messenger adenosine 3′ : 5′-cyclic monosphosphate (cyclicAMP) (Felder *et al*., 1984; Alkadhi *et al*., 1986), while D_2-like receptor activation mediates the inhibition of neurotransmitter release from sympathetic nerve terminals which in turn promotes vascular relaxation (Langer, 1980; Lokhandwala & Steenberg, 1984; Hieble *et al*., 1985).

More recently some authors have also described the presence of postjunctional D_2-like receptors in the intimal layer of mesenteric (Missale *et al*., 1985; 1988; Ricci & Amenta, 1990), jejunal (Münch *et al*., 1991), renal and glomerular vessels (Missale *et al*., 1985; Carey *et al*., 1990; Siragy *et al*., 1990). These receptors are negatively coupled to the AC but the functional significance has not been clearly defined yet. In the renal vascular endothelium and in the glomerulus the possible role of these receptors mediating a vasoconstrictor effect was suggested (Carey *et al*., 1990; Siragy *et al*., 1990) through antagonist studies with the selective D_2-like antagonist YM 09151-2, while in rabbit jejunal arteries and rat renal vascular bed, a vasodilating D_2-like receptor was suggested (Münch *et al*., 1991; Barthelmebs *et al*., 1991).

The aim of the present work was to investigate the presence of a postjunctional D_2-like receptor negatively coupled to AC which could counteract the vasorelaxant D_1-like mediated activity of the rabbit vasculature. For this purpose we used the isolated rabbit splenic artery, which is a model widely used in the study of *in vitro* D_1-like activity of dopamine receptor agonists (Hilditch & Drew, 1981; Semeraro *et al*., 1990), and which we have further developed using the superfusion system (Ferlenga *et al*., 1995).

These experiments were carried out with dopamine as internal standard, the selective D_1-like agonists fenoldopam and SKF 82958, the selective D_2-like agonist SKF 89124 and the mixed D_1/D_2-like agonists epinine and (−)-DP-5,6-ADTN testing them before and after the selective D_2-like antagonist YM 09151-2 (Terai *et al*., 1983).

In order to have an idea of the localization of these D_2-like dopamine receptors inside the rabbit splenic artery, we performed our experiments both in endothelium-denuded and endothelium-intact preparations.

In addition, experiments were also performed to evaluate the potential role of cyclicAMP in the mediation of D_2-like receptor-induced responses, evaluating the effect of the selective D_2-like agonist SKF 89124 on resting tone and on the forskolin-induced vasorelaxation.

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Methods

Superfused rabbit splenic artery preparation

Male New Zealand rabbits weighing 2.5–3 kg were killed by CO_2 inhalation and exsanguinated. The splenic artery was isolated, trimmed of adhering fat and connective tissue, and then cut into 2–3 mm long rings. Care was taken to keep the endothelium of the artery intact, while the endothelium was gently removed from some segments by inserting a steel rod and rolling it gently. These preparations were mounted in a glass superfusion chamber between two L-formed stainless steel wires and superfused at the constant flow rate of 2 ml min^{-1} through a peristaltic perfusion pump (Gilson Minipuls 3). This system is a modification of that developed by Coleman & Nials (1989).

The superfusion fluid, oxygenated (95% O_2 + 5% CO_2) and maintained at 37 ± 0.5°C, was a Krebs-Henseleit solution (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2 and glucose 11.1).

(−) Propranolol (1 μM), prazosin (1 μM), idazoxan (1 μM), indomethacin (5 μM) and EDTA (10 μM) were added to the solution in order to block β- and α-adrenoceptors, inhibit endogenous prostanoids and to prevent catecholamine oxidation, respectively.

Contractile force was recorded through a force displacement transducer (Grass FT03) on a polygraph (Gould RS3800) via a transducer amplifier Gould.

Resting tension was adjusted to approximately 2 g and the preparations submitted to a 60 min equilibration period. After this period the preparations were stabilized at 1.2 ± 0.08 g, (n = 78). They were then contracted with the thromboxane A_2 mimetic U46619 (30 nM) and once a stable contracture was reached, each preparation was submitted to a cumulative concentration-response curve to dopamine followed by washout. After the basal contraction was restored, cumulative concentration-response curves for a test compound were performed before and after 60 min incubation with the D_2-like antagonist YM 09151-2 (1 nM) or vehicle. At the end of these experiments, the integrity of the endothelium was assessed by examining the relaxant response to the endothelium-dependent vasodilator acetylcholine (10 μM). Tissues which relaxed by more than 70% or less than 15% to ACh were considered endothelium-intact and endothelium-denuded, respectively. Three mixed D_1/D_2-like agonists (dopamine, epinine and (−)-DP-5,6-ADTN), two selective D_1-like agonists (fenoldopam and SKF 82958) and the selective D_2-like agonist SKF 89124 were tested in this set of experiments.

Another set of experiments was performed in order to evaluate the potential role of cyclicAMP in the mediation of D_2-like receptor-induced responses. The effect of the administration of cumulative concentrations of the selective D_2-like agonist SKF 89124 was evaluated on rabbit superfused splenic artery at resting tone; in addition, cumulative concentration-response curves for forskolin were performed before and after 60 min incubation with the same D_2-like agonist (0.1 and 0.3 μM) or vehicle.

Cumulative concentration-response curves were obtained with a multichannel syringe infusion pump (Harvard 22), infusing a 100 fold concentrated drug into the superfusion flow at a speed of 1/300 to 1/10 that of the superfusion rate, while the single administrations were infused at a rate of 1/100 that of the superfusion rate. These infusion speeds were previously tested with vehicles and were devoid of any intrinsic effects.

Data presentation and statistical analysis

Relaxations produced by agonists were measured as changes in tension from the maximum U46619-induced contracture (i.e. the tension immediately before agonist administration) and expressed as a percentage of U46619 maximum as means ± s.e. Concentration-effect curve data were fitted by means of a nonlinear regression analysis to a Boltzman function by use of computer-assisted software ('Microcal Origin'). Agonist potency was expressed in terms of -log EC_{50} (pD_2), where EC_{50} represented the molar concentration necessary to produce 50% of the maximum agonist response.

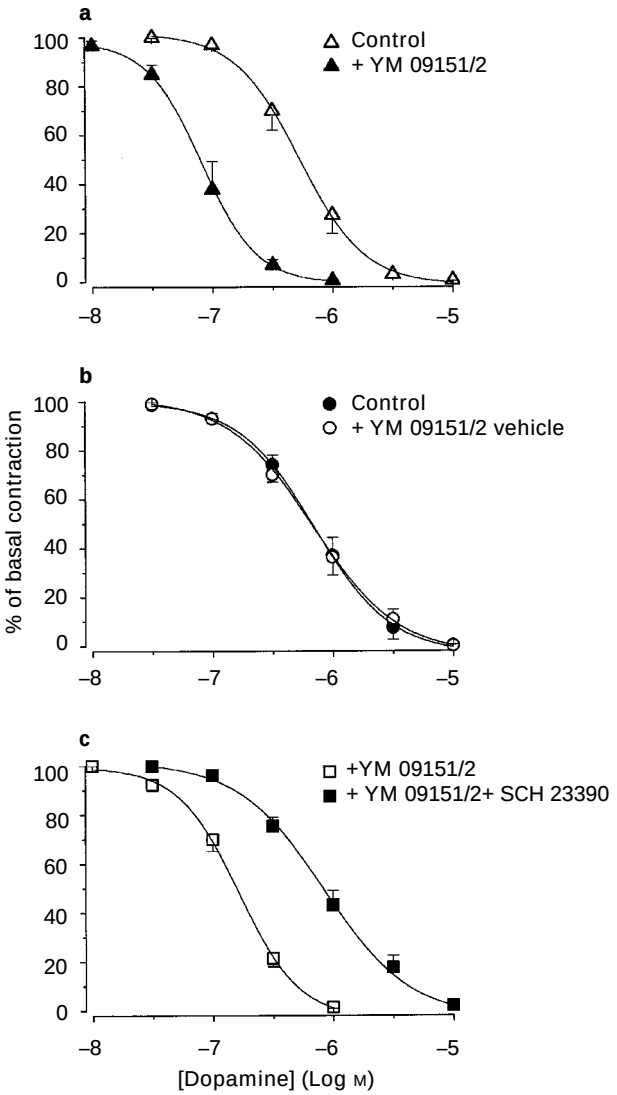

Figure 1 (a) Effect of the selective D_2-like antagonist, YM 09151-2 (1 nM), on the vasorelaxant activity of dopamine in rabbit superfused splenic artery contracted with the thromboxane A_2 mimetic U46619 (30 nM). Each data point represents the mean, and vertical lines show s.e.mean of 5 independent experiments. (b) Cumulative concentration-response curves for dopamine showing the reproductivity of its vasorelaxant activity after YM 09151-2 vehicle in U46619- contracted rabbit superfused splenic artery. Each data point represents the mean, and vertical lines show s.e.mean of 7 independent experiments. (c) Effect of the selective D_1-like antagonist, SCH 23390 (0.1 nM), on the vasorelaxant activity of dopamine in rabbit superfused splenic artery contracted with the thromboxane A_2 mimetic U46619 (30 nM). The experiments were in presence of YM 09151-2 (1 nM). Each data point represents the mean, and vertical lines show s.e.mean of 6 independent experiments.

Analysis of variance (ANOVA) according to a split-plot factorial design was performed on pD_2 values in order to evaluate the relative effect of two different conditions of treatment (before/after YM 09151-2) in two different tissue conditions (presence/absence of endothelium). A probability of 0.05 or less was considered significant.

In addition, pD_2 values obtained in presence of the D_2-like receptor antagonist YM 09151-2 (or in the presence of SKF 89124 for forskolin experiments) were compared with pD_2 values obtained in absence of the antagonist; the significance of the difference between the two mean values was determined by a paired Student's *t* test.

A probability of 0.05 or less was considered significant.

Drugs

Sources of compounds used were as follows: dopamine hydrochloride, prazosin hydrochloride, U46619 (9,11,dideoxy-11α-9α-epoxy-methano-prostaglandin $F_{2\alpha}$), dimethylsulphoxide (DMSO), indomethacin, and (−)-propranolol hydrochloride (Sigma-Aldrich, Italy); idazoxan (Reckitt & Colman, Kingston-upon-Hull, UK); EDTA (Carlo Erba, Italy); SKF 82958 (6-chloro-7,8-dihydroxy-3 allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide) (Research Biochemicals International, Natick, U.S.A.); YM 09151-2 (*cis*-N-(1-benzyl-2-methylpyrrolidin-3yl)-5-chloro-2-methoxy-4-methylaminobenzamide): a gift from Yamanouchi

Table 1 Differences between pD_2 values obtained in the absence and presence of YM 09151-2 in endothelium-intact (+) and endothelium-denuded (–) preparations

Endothelium	*% relaxation to ACh*	(n)	*Drugs*	*Difference between pD_2s*	P
(+)	75.3±2.10	(16)	Dopamine	0.68±0.042	NS
(−)	0.93±1.97	(18)		0.65±0.053	
(+)	80.2±3.68	(4)	Epinine	0.42±0.158	NS
(−)	6.8±2.76	(4)		0.40±0.031	
(+)	73.1±4.05	(4)	(−)DP-5, 6-ADTN	0.57±0.045	NS
(−)	−1.1±2.10	(4)		0.69±0.224	
(+)	70.8±3.15	(5)	Fenoldopam	0.07±0.091	NS
(−)	−1.6±3.11	(6)		0.11±0.112	
(+)	77.2±3.05	(3)	SKF 82958	0.16±0.075	NS
(−)	−0.4±1.80	(4)		0.18±0.091	
(+)	82.8±2.02	(6)	SKF 89124	ND	
(−)	12.8±1.97	(4)		ND	

The size of the relaxant effect to acetylcholine (ACh) 10 μM indicated the endothelium condition. Values shown are means±s.e. NS: the difference between the differences of pD_2 values obtained in absence and in presence of YM 09151-2 in endothelium-intact vs endothelium-denuded preparations was not significant ($P>0.05$, ANOVA). ND: not determined

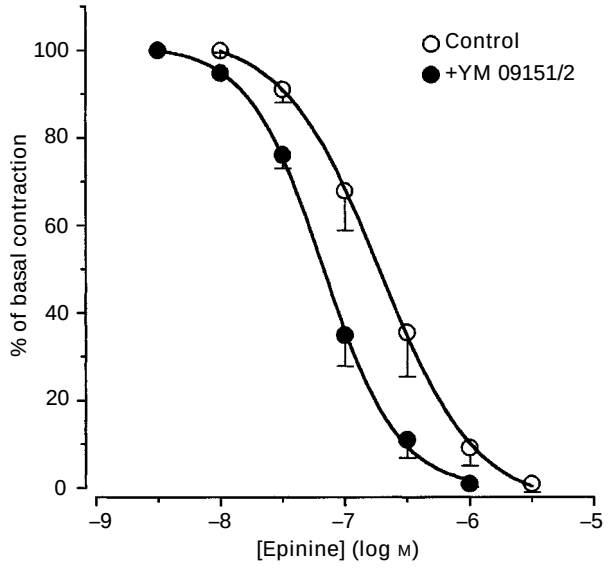

Figure 2 Effect of the selective D_2-like antagonist, YM 09151-2 (1 nM), on the vasorelaxant activity of the mixed D_1/D_2-like agonist epinine in rabbit superfused splenic artery contracted with the thromboxane A_2 mimetic U46619 (30 nM). Each data point represents the mean, and vertical lines show s.e.mean of 8 independent experiments.

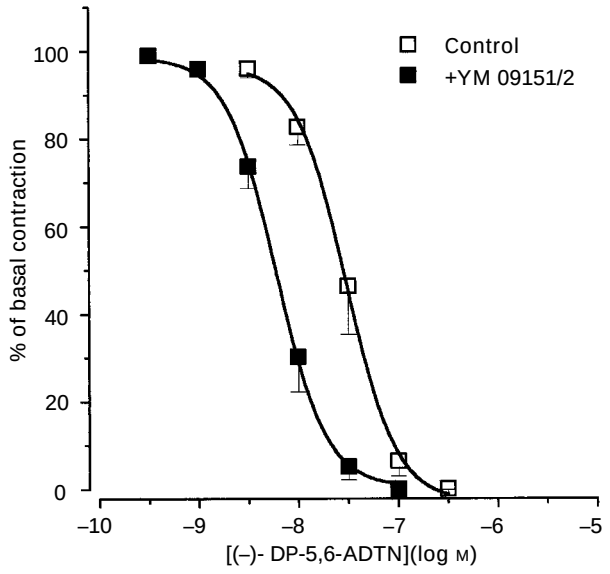

Figure 3 Effect of the selective D_2-like antagonist, YM 09151-2 (1 nM), on the vasorelaxant activity of the mixed D_1/D_2-like agonist (−)-DP-5,6-ADTN in rabbit superfused splenic artery contracted with the thromboxane A_2 mimetic U46619 (30 nM). Each data point represents the mean, and vertical lines show s.e.mean of 8 independent experiments.

Pharmaceutical Co. Ltd., Japan; SKF 89124 (4-[N,N-di-n-propylamino)ethyl]-7-hydroxy-2(3H)indolone), (−)DP-5,6-ADTN ((−)-2-dipropylamino-5,6-dihydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide), fenoldopam (6-chloro-7, 8-dihydroxy-1-(p-hydroxyphenyl(-2,3,4,5-tetrahydro-(1H)-3-benzazepine) and epinine were all synthesized by the Department of Chemistry (Zambon Group, R&D Division, Bresso).

U46619, purchased as methyl acetate solution (10 mg ml^{-1}), was dissolved in 0.3% $NaHCO_3$ to give a stock solution (100 μg ml^{-1}) kept at −20°C; further dilutions were performed in 0.3% $NaHCO_3$. EDTA, (−)-propranolol hydrochloride and idazoxan were dissolved in distilled water. Dopamine, epinine, fenoldopam, (−)-DP-5,6-ADTN and SKF 89124 were dissolved in acqueous sodium metabisulphite (0.1%) solution just before use. Prazosin, YM 09151-2 and SKF 82958 were dissolved initially in 100% DMSO and subsequently diluted in distilled water. Indomethacin was dissolved and diluted in stoichiometric, aqueous 2 N NaOH solution.

Results

In a representative series of experiments the tension generated in response to U46619 (30 nM) was 5.17±0.18 g ($n=50$) and represented approximately 80% of the maximal U46619 effect in this preparation.

The D_2-like antagonist YM 09151-2 (1 nM) induced a significant ($P<0.01$) leftward shift of the concentration-response curve to dopamine, the pD_2 value changed from 6.27±0.09 to 7.11±0.10 (Figure 1a), whereas no differences in the vasorelaxant activity of dopamine were seen before and after vehicle ($pD_2=6.18\pm0.11$ and 6.15±0.11, respectively, Figure 1b).

The dopamine vasorelaxant activity observed in our experimental conditions (in the presence of YM 09151-2 1 nM) was antagonized by the selective D_1-like antagonist SCH 23390 (0.1 nM) (pD_2 before = 6.81±0.05, pD_2 after = 6.09±0.09), confirming a D_1-like mediated vasorelaxant effect (Figure 1c).

The D_2-like antagonist YM 09151-2 induced the same degree of effect with all of the substances tested in both endothelium-denuded and endothelium-intact preparations (Table 1); for this reason, the results presented in the figures are the mean of all the experiments.

Epinine, like dopamine, was able to reverse completely the U46619-induced contracture showing a $pD_2=6.73\pm0.13$; the treatment with YM 09151-2 induced a significant ($P<0.05$) increase in the epinine vasorelaxant activity ($pD_2=7.14\pm0.02$) (Figure 2).

No differences in the vasorelaxant activity of epinine were seen before and after vehicle ($pD_2=6.78\pm0.12$ and 6.80±0.08, respectively, $n=7$).

As can be seen in Figure 3, the vasorelaxant activity of (−)-DP-5,6-ADTN was also significantly ($P<0.01$) increased by the D_2-like antagonist YM 09151-2, the pD_2 value increasing from 7.56±0.09 to 8.19±0.09; the concentration-response curve of (−)-DP-5,6-ADTN remained unchanged after vehicle (pD_2 before = 7.65±0.07, pD_2 after = 7.72±0.10, $n=8$).

YM 09151-2 did not modify the partial ($\alpha=0.65\pm0.06$) vasorelaxant activity of the selective D_1-like agonist fenoldopam (pD_2 before = 8.31±0.09; pD_2 after = 8.40±0.09), whereas dopamine activity in these preparations was complete and significantly ($P<0.01$) improved by the D_2-like antagonist YM 09151-2 (pD_2 before = 6.33±0.07, pD_2 after = 6.95±0.04) (Figure 4).

Also for this compound we observed a reproducibility of the concentration-response curve after vehicle (pD_2 before = 8.26±0.13; pD_2 after = 8.37±0.11, $n=7$), as well as for the control dopamine (pD_2 before = 6.31±0.09; pD_2 after = 6.39±0.07, $n=7$).

In contrast to fenoldopam, the other D_1-like agonist tested, SKF 82958 (Figure 5), was able to reverse completely the U46619-induced contracture ($pD_2=8.55\pm0.10$). However, with this compound, we did not observe any improvement after YM 09151-2 treatment ($pD_2=8.58\pm0.08$). Also for this set of experiments we observed a significant ($P<0.01$) leftward

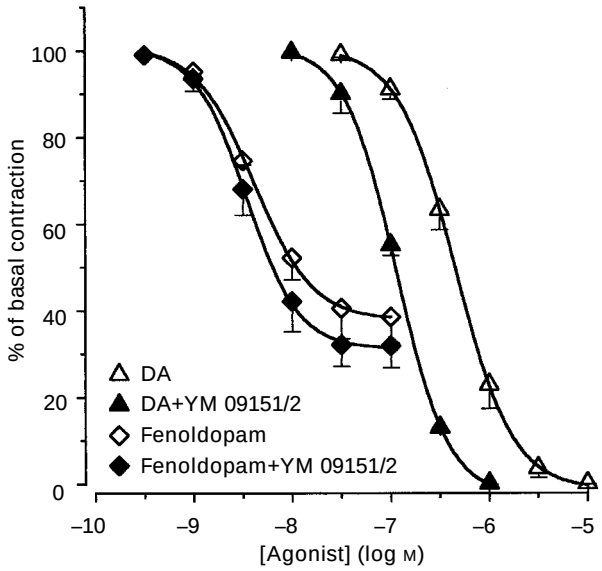

Figure 4 Effect of the selective D_2-like antagonist, YM 09151-2 (1 nM), on the vasorelaxant activities of dopamine (DA) and fenoldopam, a selective D_1-like agonist, in rabbit superfused splenic artery contracted with the thromboxane A_2 mimetic U46619 (30 nM). Each data point represents the mean, and vertical lines show s.e.mean of 11 independent experiments.

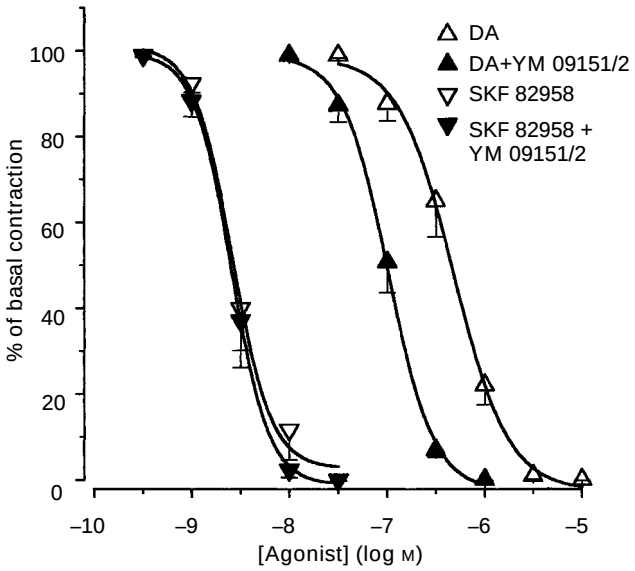

Figure 5 Effect of the selective D_2-like antagonist, YM 09151-2 (1 nM), on the vasorelaxant activities of dopamine (DA) and SKF 82958, a selective D_1-like agonist, in rabbit superfused splenic artery contracted with the thromboxane A_2 mimetic U46619 (30 nM). Each data point represents the mean, and vertical lines show s.e.mean of 7 independent experiments.

shift of the concentration-response curve of the internal dopamine in the presence of the D_2-like antagonist (pD_2 before = 6.35 ± 0.09; pD_2 after = 7.01 ± 0.07).

No differences in the vasorelaxant activity of SKF 82958 and dopamine were seen before and after vehicle (pD_2 before = 8.49 ± 0.07; pD_2 after = 8.66 ± 0.04 and pD_2 before = 6.39 ± 0.12; pD_2 after = 6.30 ± 0.12, respectively, $n = 6$).

The D_2-like agonist SKF 89124 (Figure 6), *per se*, was not able to produce any vasorelaxant effect up to 10 μM, unlike dopamine on the same preparations ($pD_2 = 6.01 \pm 0.05$), but in the presence of YM 09151-2, SKF 89124 induced a 40% relaxation at 10 μM, a concentration at which the compound probably begins to interact with D_1-like receptors (Hieble *et al*., 1989).

No differences in the activity of SKF 89124 and dopamine were seen before and after vehicle (SKF 89124: no relaxation; dopamine pD_2 before = 6.18 ± 0.11; pD_2 after = 6.16 ± 0.09, $n = 4$).

Interestingly, the D_2-like agonist SKF 89124, did not modify the basal tension up to 100 μM (basal tension: 1.18 ± 0.17 g, tension after SKF 89124 100 μM: 1.21 ± 0.15, $n = 4$), whereas it was able to attenuate the forskolin-induced vasorelaxation in U46619-precontracted rings. As shown in Figure 7a, SKF 89124 (0.1 μM) reduced the forskolin-induced relaxation (pD_2 before = 7.28 ± 0.07; pD_2 after = 7.09 ± 0.06) while the compound, at the concentration of 0.3 μM, induced a significant ($P < 0.01$) rightward shift of the forskolin concentration-response curve (pD_2 before = 7.31 ± 0.02; pD_2 after = 7.08 ± 0.02, Figure 7b). No differences in the activity of forskolin were seen before and after vehicle (pD_2 before = 7.49 ± 0.13; pD_2 after = 7.39 ± 0.12, Figure 7c).

Discussion

Two dopamine receptors have been characterized in the periphery: postjunctional D_1-like receptors and neuronal D_2-like receptors.

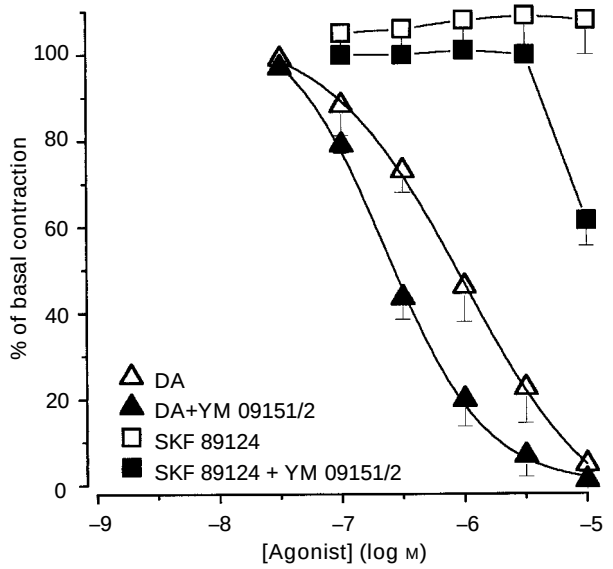

Figure 6 Effect of the selective D_2-like antagonist, YM 09151-2 (1 nM), on the vasorelaxant activities of dopamine (DA) and SKF 89124, a selective D_2-like agonist, in rabbit superfused splenic artery contracted with the thromboxane A_2 mimetic U46619 (30 nM). Each data point represents the mean, and vertical lines show s.e.mean of 12 independent experiments.

In addition to these two dopamine receptors, the presence and above all the functional significance of postjunctional D_2-like receptors in peripheral vascular tissues is under discussion. This is due to the extremely variable results obtained in different animal models, raising the possibility that differences

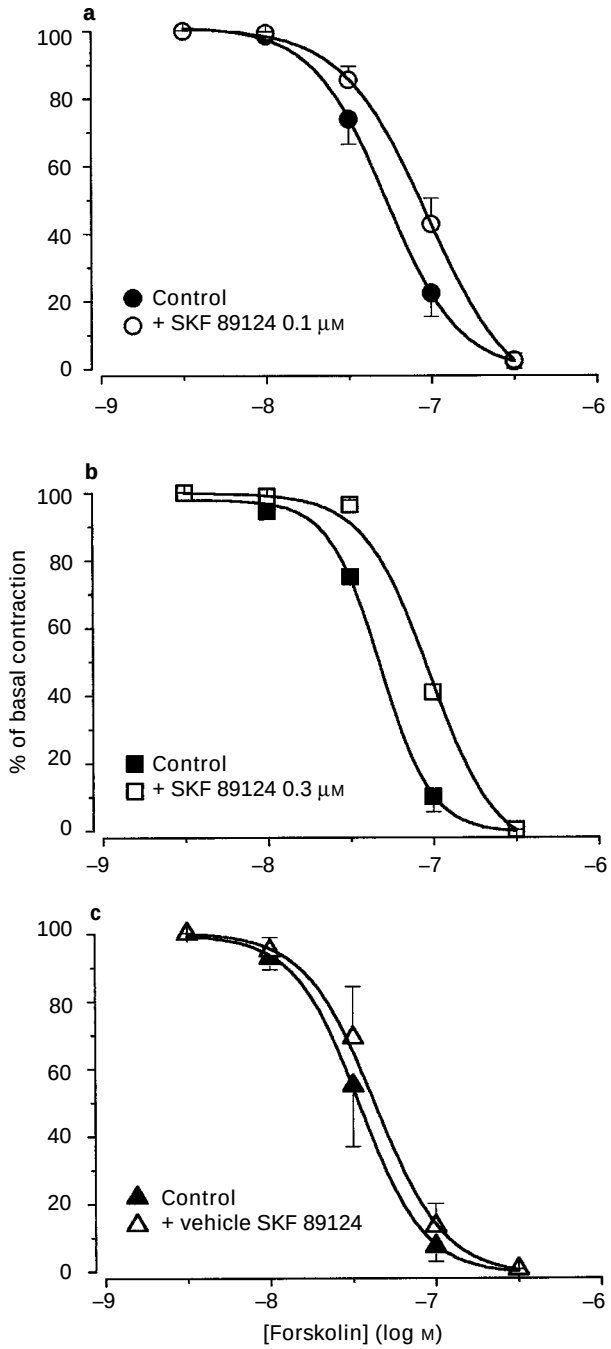

Figure 7 (a) Effect of the selective D_2-like agonist, SKF 89124 (0.1 μM), on the vasorelaxant activity of forskolin in rabbit superfused splenic artery contracted with the thromboxane A_2 mimetic U46619 (30 nM). Each data point represents the mean, and vertical lines show s.e.mean of 6 independent experiments. (b) Effect of the selective D_2-like agonist, SKF 89124 (0.3 μM), on the vasorelaxant activity of forskolin in rabbit superfused splenic artery contracted with the thromboxane A_2 mimetic U46619 (30 nM). Each data point represents the mean, and vertical lines show s.e.mean of 3 independent experiments. (c) Cumulative concentration-response curves for forskolin showing the reproductivity of its vasorelaxant activity after SKF 89124 vehicle in U46619-contracted rabbit superfused splenic artery. Each data point represents the mean, and vertical lines show s.e.mean of 3 independent experiments.

between animal species could exist. For example it is interesting to note that whereas bromocriptine does not and quinpirole does increase renal blood flow in the dog (Jose *et al*., 1986; Horn & Kohli, 1991) the opposite was seen by Barthelmebs *et al*. (1991) in the rat kidney, while Schmidt *et al*. (1982, 1987) observed vasodilatation in the rat kidney with both ergolines.

However, the *in vivo* results may be influenced by various factors, such as differences in plasma protein binding, distribution and metabolism which make it difficult to evaluate the receptor affinity of different agonists and antagonists in equilibrium condition.

As indicated in the Introduction, recently some authors have also demonstrated the presence of postjunctional D_2-like receptors in the intimal layer of mesenteric (Missale *et al*., 1985; 1988; Ricci & Amenta, 1990), jejunal (Münch *et al*., 1991), renal and glomerular vessels (Missale *et al*., 1985; Carey *et al*., 1990; Siragy *et al*., 1990) utilizing two other experimental approaches: radioligand binding studies and biochemical studies with adenylate cyclase as a marker. According to the biochemical measurements, these receptors decreased or had no effect on AC activity, but the functional significance has not yet been clearly defined.

There is thus a clear need to study dopamine agents in isolated tissue preparations in order to measure affinity and functional response and to gain more knowledge of the differences between these receptors. Nevertheless, even in these isolated preparations various problems have been encountered which make it difficult to compare the results, mainly due to the absence of specific dopamine agents and to the different experimental conditions adopted.

Rabbit splenic artery has been widely used in the study of the vascular D_1-like receptor: in this preparation precontracted with the thromboxane A_2 mimetic U46619, D_1-like agonists are able to induce a cyclicAMP-mediated relaxation (Hilditch & Drew, 1981; Clark *et al*., 1989). However, extensive experience has shown that dopamine produces little or no relaxation at all in about 60% of preparations (Clark *et al*., 1989). An inefficient receptor coupling mechanism or a different receptor density from tissue to tissue has been hypothesized to explain this variability.

Our work showed clearly that the insensitivity of many preparations to dopamine and dopamine agonists may be accounted for by a counteracting activity mediated by postjunctional D_2-like receptors. To demonstrate the presence of a counteracting D_2-like activity linked to an inhibition of AC better, the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX), suggested by Clark *et al*., (1989) to improve D_1-like mediated relaxation, was removed from the Krebs solution in order to normalize the interaction of the receptors with AC. Similarly, the α-blocking agent phenoxybenzamine, which has been shown to interact with dopamine receptors (Walton *et al*., 1978; Hall *et al*., 1993; Van der Graaf *et al*., 1995) was substituted by prazosin and idazoxan, α_1- and α_2-selective antagonists respectively.

The results which we have obtained, showing that a D_2-like antagonist, YM 09151-2, enhances the relaxing response of dopamine and mixed D_1/D_2-like dopamine agonists but not the activity of selective D_1-like agonists, indicates the presence in this preparation of a D_2-like receptor counteracting the relaxing effect of a non selective dopamine compound.

In contrast to the splenic vasodilatation engendered by selective D_1-like receptor stimulation with agonists, blockade of the D_2-like receptors with YM 09151-2 *per se* did not induce vasoconstriction, but instead was able to improve the D_1-like mediated response. On the other hand, we were not able to induce a D_2-like mediated contracture of the rabbit splenic artery with a specific D_2-like receptor agonist, since SKF 89124 up to 100 μM was inactive on resting tone.

In the present study, forskolin, which has been shown to increase cellular cyclicAMP directly by acting on a catalytic site of AC (Seamon & Daly, 1981), produced a concentration-related vasorelaxation that was attenuated by the selective D_2-like agonist SKF 89124.

Such a result was also obtained in anaesthetized rats by Sakamoto *et al*. (1994); these authors observed an attenuation by SKF 89124 of adenylate cyclase-induced increases (through infusion of IBMX or forskolin) in renal sodium excretion.

The results we obtained indicate that activation of postjunctional D_2-like receptors opposes cyclicAMP-mediated effector response via an inhibitory influence on adenylate cyclase. It is likely that under basal conditions the involvement of the cyclicAMP-generating system in the maintenance of vascular tone is minimal. Therefore, activation of D_2-like postjunctional receptors, which are negatively coupled to adenylate cyclase, does not cause significant alteration in the vascular tone. However, when the activity of the cyclicAMP-generating system is enhanced, the inhibitory influence from activation of D_2-like receptors becomes manifest.

As regards their location inside rabbit splenic artery, it is possible to hypothesize an extra-endothelial location because the D_2-like antagonist YM 09151-2 induced the same degree of effect with all the tested substances in both endothelium-denuded and endothelium-intact preparations. Whether this D_2-like receptor is situated on the intimal layer, as suggested by Ricci & Amenta (1990) in the rat mesenteric vascular tree, or in other sites of the vessel must be further investigated.

In summary, it is possible to hypothesize the presence in this preparation of a D_2-like postjunctional receptor counteracting the cyclicAMP-mediated D_1-like relaxing effect.

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