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Renal vasodilatation by dopexamine and fenoldopam due to α_1-adrenoceptor blockade

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1 The renal vascular responses of the rat isolated perfused kidney to the dopamine D_1-receptor agonists, dopexamine and fenoldopam, were examined.

2 Both kidneys were perfused *in situ* at constant flow rate (11 ml min^{-1}) with Krebs-bicarbonate solution at 37°C. The perfusion pressure was monitored and to enable vasodilator responses to be measured, the resting perfusion pressure was raised by infusing noradrenaline (6×10^{-9} M).

3 Dose-related vasodilator responses to bolus doses of dopexamine and fenoldopam were obtained. However, these were not antagonized by the D_1-receptor antagonist, SCH 23390, indicating that D_1-receptors were not involved.

4 Bolus doses of the α_1-adrenoceptor antagonist, prazosin, caused similar dose-related vasodilator responses indicating the possibility that α_1-adrenoceptor blocking properties of dopexamine and fenoldopam were responsible for the vasodilatation.

5 α-Adrenoceptor blockade by dopexamine and fenoldopam was confirmed by the parallel displacement of dose-response curves for the vasopressor responses to noradrenaline. pA_2 values were determined by Schild analysis for dopexamine, fenoldopam and prazosin antagonism of noradrenaline in the presence of neuronal (cocaine, 10^{-5} M) and extraneuronal uptake blockade (metanephrine, 10^{-5} M). The values were 6.23, 6.02 and 8.91, respectively. Schild plot slopes of unity were obtained for dopexamine and fenoldopam indicating competitive antagonism. A slope of greater than unity for prazosin may be explained by the lack of equilibrium conditions associated with bolus doses of noradrenaline, the responses of which are affected more by the high affinity antagonist, prazosin, than the two lower affinity antagonists.

6 This study has demonstrated that renal vasodilator responses to the D_1-receptor agonists, dopexamine and fenoldopam, are due to a brief antagonism of the α-adrenoceptor-mediated vasoconstriction induced by noradrenaline. This presumably masks any direct D_1-receptor-mediated vasodilatation.

Keywords: Rat perfused kidneys; vasodilatation; noradrenaline-induced vasoconstrictor tone; dopexamine; fenoldopam; α-adrenoceptor blockade

Introduction

The antihypertensive drug fenoldopam was introduced as a potent, selective renal vasodilator, acting predominantly through stimulation of renal vascular D_1-receptors. Unlike dopamine, it lacks α-adrenoceptor agonist activity (Hahn *et al.*, 1982). Similarly, dopexamine, used in the acute management of low cardiac output conditions, also produces renal vasodilatation by selective stimulation of renal vascular D_1-receptors and lacks α_1-adrenoceptor agonist activity. In addition, the β_2-adrenoceptor agonist activity of this drug promotes reduction of afterload due to vasodilatation and mild positive inotropy (Smith & O'Connor 1988). In the present study, the effects of both drugs were examined in the rat isolated perfused kidney. This preparation has been used as a model for D_1-dopamine receptor-mediated vasodilatation. There are several reports of dopamine and selective D_1-receptor agonists, including fenoldopam, producing falls in perfusion pressure when the basal pressure is raised by vasoconstrictors including prostaglandin $F_{2\alpha}$ (Imbs *et al.*, 1984; Schmidt *et al.*, 1987). However, this paper describes an unexpected observation that although both drugs cause vasodilatation of the rat renal vasculature, constricted with noradrenaline, it is not due to D_1-receptor agonist activity but to α_1-adrenoceptor blockade. This property of the two drugs is then quantified by determination of pA_2 values and compared to prazosin.

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Methods

Tissue preparation

Male Wistar rats (300-350 g) were anaesthetized with pentobarbitone sodium (60 mg kg^{-1}, i.p.). The abdomen was opened and a polythene cannula was advanced retrogradely along the abdominal aorta until the tip lay opposite the left renal artery. The mesenteric artery was tied with a cotton ligature and the aorta was ligated immediately below the coeliac artery. Both kidneys were then perfused, at a flow rate of approximately 11 ml min^{-1}, with carboxygenated Krebs-bicarbonate solution at 37°C by means of a Watson-Marlow peristaltic pump (type 502S). The Krebs-bicarbonate solution had the following composition (mM): NaCl 118.4, $NaHCO_3$ 24.9, KCl 4.7, $CaCl_2$ 1.9 (used as the dihydrate), $MgSO_4$ 1.15 (as the heptahydrate), KH_2PO_4 1.15 and glucose 11.7. Ascorbic acid (1 mM) was added to reduce the oxidation of noradrenaline. Although earlier studies have employed a more complex medium for examining renal function in isolated perfused kidneys, this was not essential for the present vascular studies. No deleterious effects were observed upon the responses to noradrenaline over the time period of each experiment.

The vena cava was cut between the left and right renal veins to allow escape of perfusate. Alterations in perfusion pressure, arising from changes in renal vascular resistance, were recorded on a Lectromed polygraph (MT8 P, Welwyn Garden City, Hertfordshire) by means of a pressure transducer (Bell and Howell, type 4-327-L221) situated between the perfusion cannula and the warming coil. A Condon

manometer was also connected in series with the pressure transducer to accommodate some degree of volume change during drug responses.

Construction of dose-response curves

In order to observe substantial vasodilator responses, the renal vasculature was constricted by addition of 6×10^{-9} M noradrenaline to the perfusion medium. When the elevated perfusion pressure (213.2 ± 5.3 mmHg, $n = 6$) had stabilized (about 15 min), non-cumulative dose-response curves to vasodilator drugs were obtained by injection of increasing bolus doses into the perfusion cannula. Vasodilatation was measured as the peak reduction in perfusion pressure at each dose of drug, measured from the stable elevation of pressure induced by noradrenaline. The ED_{50} value was calculated as the dose producing 50% of maximum vasodilatation and the geometric mean with 95% confidence limits was determined.

Calculation of pA_2 values

pA_2 values for the antagonism of vasoconstrictor responses to noradrenaline by dopexamine, fenoldopam and prazosin were calculated (Arunlakshana & Schild, 1959). Following an initial non-cumulative dose-response curve to increasing bolus doses of noradrenaline in the absence of antagonist, a 30 min period of antagonist infusion was allowed before a second dose-response curve was constructed in its presence. Responses were measured as the increase in perfusion pressure. To compensate for time-dependent changes in tissue sensitivity to noradrenaline between the first and second dose-response curves, correction factors derived from time-matched control experiments in the absence of antagonist were applied to the first curve. At each dose of noradrenaline, the response ratio (Curve 2/Curve 1) was calculated for the control experiments. Mean correction ratios ($n \geq 4$) were then multiplied by the corresponding response values of the initial curve of the 'test' experiments. Dose-response curves were then plotted as a percentage of the maximum response and the ED_{50} determined as the dose producing 50% of the maximum response. Dose-ratios were calculated by division of individual ED_{50} values obtained in the presence of antagonist (Curve 2) by the corresponding values in the absence of antagonist (Curve 1). Geometric mean ED_{50} values and their 95% confidence limits were calculated. Values for pA_2 were obtained by plotting log (dose ratio -1) on the ordinate scale against log antagonist concentration on the abscissa scale; the intercept of the regression line on the abscissa scale giving the pA_2 value.

Statistics

Student's t tests were used to test for significant differences between geometric mean ED_{50} values, arithmetic mean maximum responses and pA_2 values and to test the slopes of Schild plots for significant differences from unity. Differences were considered significant with $P < 0.05$. For comparison of more than two means, Tukey's multirange test (95% probability) was used.

Drugs and solutions

Drugs were obtained from the following sources:- cocaine hydrochloride (Hillcross Pharmaceuticals, Burnley, Lancashire, U.K.), dopexamine hydrochloride (Fisons Pharmaceuticals, Loughborough, Leicestershire, U.K.), fenoldopam mesylate (SKB, Philadelphia P.A., U.S.A.), (±)-metanephrine hydrochloride (Sigma, Poole, Dorset, U.K.), (−)-noradrenaline bitartrate (Sigma), pentobarbitone sodium (Sagatal) (Rhone Merieux, Tallaght, Dublin, Ireland), prazosin hydrochloride (Pfizer, Sandwich, Kent, U.K.), (±-propranolol hydrochloride (Inderal) (ICI Pharmaceuticals, Macclesfield, Cheshire, U.K.) and SCH 23390 [(**R**)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepine] as the maleate (Schering Corporation, Bloomfield, NJ, U.S.A.). Stock solutions were prepared as follows:- dopexamine and fenoldopam were made up in 0.9% saline containing 0.05% w/v sodium metabisulphite. Prazosin, cocaine and metanephrine were dissolved in twice distilled water, the latter containing 0.1% w/v sodium metabisulphite. Noradrenaline was dissolved in 0.01 M HCl. SCH 23390 was initially dissolved in a small quantity of 2 M HCl and was then made up to the required concentration by addition of twice distilled water. Stock solutions of dopexamine were stored frozen in small aliquots for a maximum of 5 days. Stock solutions of SCH 23390 were stored in the same way for several weeks. Solutions of prazosin and noradrenaline were prepared immediately prior to the experiment. Working drug concentrations were obtained by serial dilution of stock solutions in Krebs-bicarbonate solution.

Results

Vasodilator responses to dopexamine and fenoldopam

Responses to dopexamine were examined in the presence of β-adrenoceptor blockade by propranolol (10^{-6} M). Both fenoldopam and dopexamine caused dose-related falls in perfusion pressure (Figure 1). The maximum response to dopexamine was consistently greater than that of fenoldopam

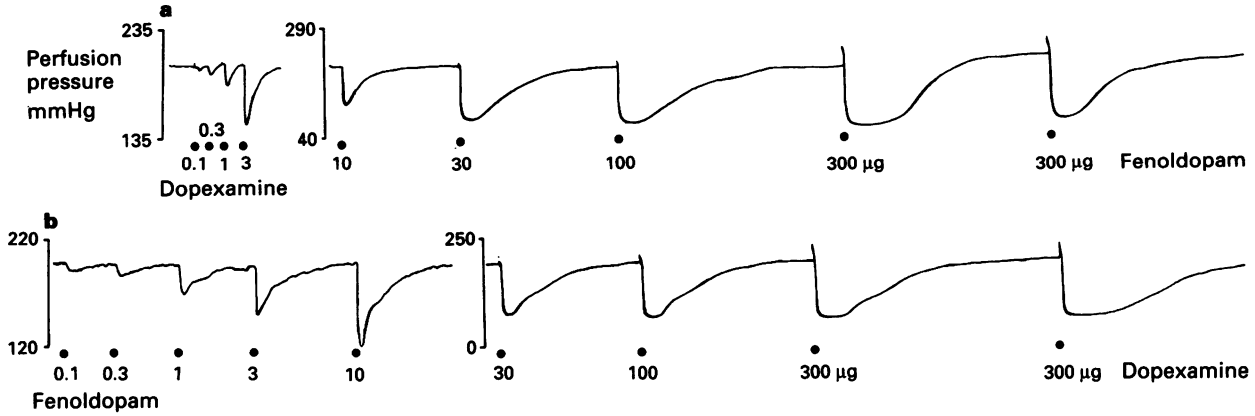

Figure 1 Typical vasodilator responses to dopexamine (a) and fenoldopam (b) in rat isolated perfused kidneys. Traces show the effect of a 300 µg dose of dopexamine following a dose-response curve to dopexamine (a) and *vice versa* (b). Time scale; 10 min between 10 and 30 µg doses of fenoldopam.

(Figure 2). This was shown by administration of a maximum dose of one agonist following completion of the dose-response curve to the other, as illustrated in Figure 1.

Effect of SCH 23390 on responses to dopexamine and fenoldopam

The dose-response curves for vasodilatation in the noradrenaline-constricted kidney preparation in response to bolus doses of dopexamine and fenoldopam were not significantly dextrally displaced in the presence of the competitive D_1-receptor antagonist, SCH 23390 (10^{-6} M) (Figure 3). The basal noradrenaline-induced perfused pressure prior to administration of the dopamine agonist was not affected by the presence of SCH 23390. The mean values prior to dopexamine of 205.5 ± 5.1 and 216.3 ± 4.7 mmHg ($n = 4$) in the absence and presence of SCH 23390, respectively, were not significantly different ($P > 0.05$).

Effect of prazosin

As with dopexamine and fenoldopam, bolus doses of the α_1-adrenoceptor-selective competitive antagonist, prazosin (Cambridge *et al.*, 1977), caused dose-related falls in perfusion pressure (Figure 2). Although the ED_{50} for prazosin (44.8(15.2–132.0)ng) was significantly lower than for dopexamine (3.8(2.6–5.3)μg) and fenoldopam (5.9(4.6–9.7)μg), the maximum decreases in perfusion pressure caused by fenoldopam (122.5 ± 6.3 mmHg) and dopexamine (127.1 ± 5.3 mmHg) were significantly greater than the maximum for prazosin (94.0 ± 2.6 mmHg).

Effects of dopexamine, fenoldopam and prazosin on noradrenaline-induced vasoconstriction

Concentration-response curves for noradrenaline were progressively displaced to the right by dopexamine (Figure 4), fenoldopam (Figure 5) and prazosin. pA_2 values for this antagonism by dopexamine and fenoldopam were determined either in the presence or absence of neuronal and extraneuronal uptake block by cocaine (10^{-5} M) and metanephrine (10^{-5} M), respectively. The pA_2 for prazosin was obtained in the presence of neuronal and extraneuronal uptake block but not in their absence.

In the presence of uptake block, the Schild plots for the antagonism of the vasoconstrictor responses of noradrenaline by dopexamine and fenoldopam (Figure 6a and b) had slopes which were not significantly different from unity (Table 1). The pA_2 value obtained for dopexamine was (6.23 ± 0.11) and a similar value was determined for fenoldopam (6.02 ± 0.21). The antagonism of noradrenaline responses by prazosin yielded a pA_2 of 8.91 ± 0.58, although the slope of the Schild regression (1.28 ± 0.16) was significantly greater than unity ($P < 0.001$) (Figure 6c).

In the absence of uptake block, the slope of the Schild plot for fenoldopam (0.35 ± 0.12, Figure 6e) was significantly less than unity ($P < 0.01$). The pA_2 value (8.36 ± 0.21) was consequently higher than the corresponding value in the presence of uptake blockade. In contrast, the slope of the Schild plot for dopexamine (Figure 6d) remained close to unity (1.08 ± 0.20), resulting in a pA_2 value which was close to that obtained in the presence of uptake block (Table 1).

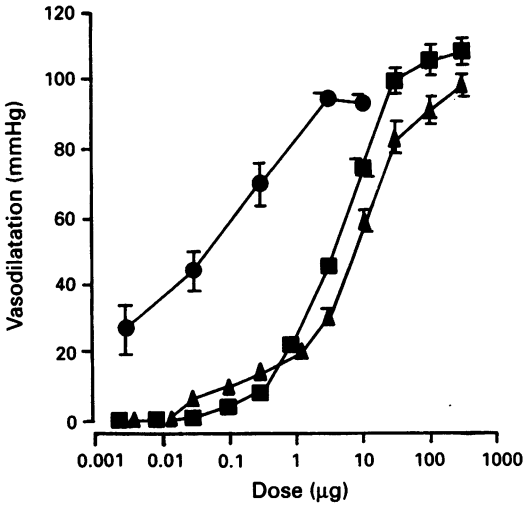

Figure 2 Comparison of the dose-response curves for vasodilatation of the noradrenaline-constricted isolated perfused kidney of the rat by prazosin (●), dopexamine (■) and fenoldopam (▲). Points are mean falls in perfusion pressure (mmHg) (± s.e.mean, $n \geq 4$).

Discussion

In view of the established D_1-receptor agonist activities of fenoldopam (Hahn *et al.*, 1982) and dopexamine (Brown *et al.*, 1985), it was assumed that their vasodilator responses in the noradrenaline-constricted isolated perfused kidney were

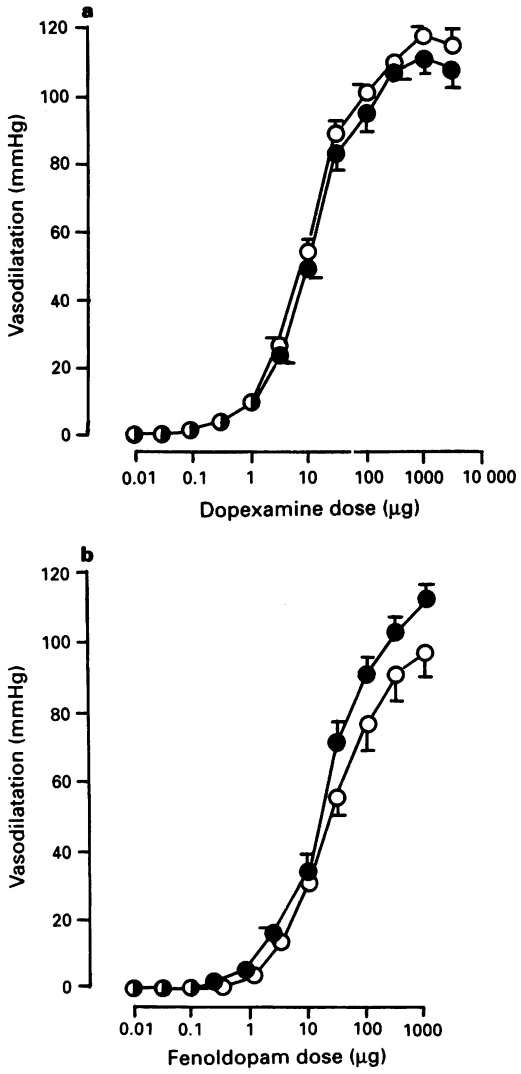

Figure 3 Effect of SCH 23390 (10^{-6} M) on the vasodilator responses of (a) dopexamine and (b) fenoldopam in the rat isolated perfused kidney constricted with noradrenaline. Curves were obtained in the absence (○) or presence (●) of SCH 23390. Curves for dopexamine were constructed in the presence of propranolol (10^{-6} M). Points are mean falls in perfusion pressure (mmHg) (± s.e.mean, $n = 4$).

the result of dopamine D_1- receptor stimulation. Furthermore, this isolated organ is known to exhibit vasodilator responses to dopamine (Imbs *et al.*, 1984; Schmidt *et al.*, 1987). However, the inability of the potent, selective D_1-receptor antagonist, SCH 23390 (Hilditch *et al.*, 1984) to antagonize the vasodilator responses to either fenoldopam or dopexamine at a concentration of 10^{-6} M, suggested that D_1-receptors were not involved. This concentration is known to be sufficient to antagonize D_1-receptor-mediated responses in this tissue (Schmidt *et al.*, 1987) and the splenic artery (Hilditch & Drew, 1985) where pA_2 values of about 10 were obtained. The possibility was considered that the responses were a result of antagonism of the α_1-adrenoceptor-mediated noradrenaline pre-constriction rather than D_1-receptor-mediated relaxation. The similarity of the fenoldopam and dopexamine responses to those produced by the competitive α_1-adrenoceptor antagonist, prazosin (Cambridge *et al.*, 1977) in the same preparation, supported this suggestion. This, together with reports in the literature demonstrating antagonism by fenoldopam of the α_1-adrenoceptor-mediated responses of noradrenaline in the rat (Ohlstein *et al.*, 1985), dog and rabbit (Nakamura *et al.*, 1986) isolated blood vessels, prompted efforts to confirm and quantify the α_1-adrenoceptor antagonist properties of fenoldopam and dopexamine in the noradrenaline-constricted rat isolated perfused kidney.

Both fenoldopam and dopexamine caused concentration-related displacement of the noradrenaline dose-response curves, indicating α_1-adrenoceptor blockade. Since dopexamine has been shown to have uptake blocking activity (Mitchell *et al.*, 1987) which may have influenced the measurement of antagonism, pA_2 values for dopexamine and fenoldopam were determined both in the presence and absence of uptake blockade.

Effect of uptake block on the antagonism of noradrenaline responses by fenoldopam

Since the slope of the Schild plot for fenoldopam was not significantly different from unity in the presence of uptake block, but was significantly less than unity in the absence of uptake block, it seems likely that the low slope obtained under the latter conditions was due to the operation of an agonist uptake mechanism rather than to a lack of competitive antagonism (Blinks, 1967; Furchgott, 1967; Langer & Trendelenburg, 1969). The low slope can be explained in terms of a model proposed by Langer and Trendelenberg (1969) which ascribed three regions to the uptake process. In

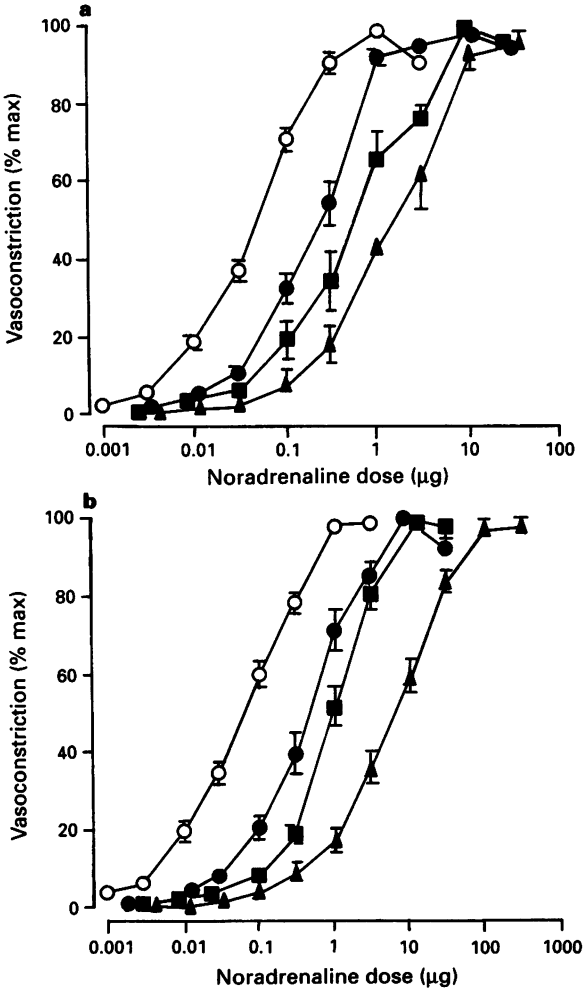

Figure 4 Effect of increasing concentrations of dopexamine on the dose-response curve for vasoconstriction of the rat isolated perfused kidney by noradrenaline in the absence (a) and presence (b) of uptake blockade by cocaine and metanephrine. Curves were obtained in the absence (○) and repeated once in the presence of dopexamine, 3×10^{-6} M (●); 10^{-5} M (■); 3×10^{-5} M (▲). Points are the mean responses expressed as a percentage of the maximum response (± s.e.mean, $n \geq 4$). Pre-antagonist curves are corrected from control experiments.

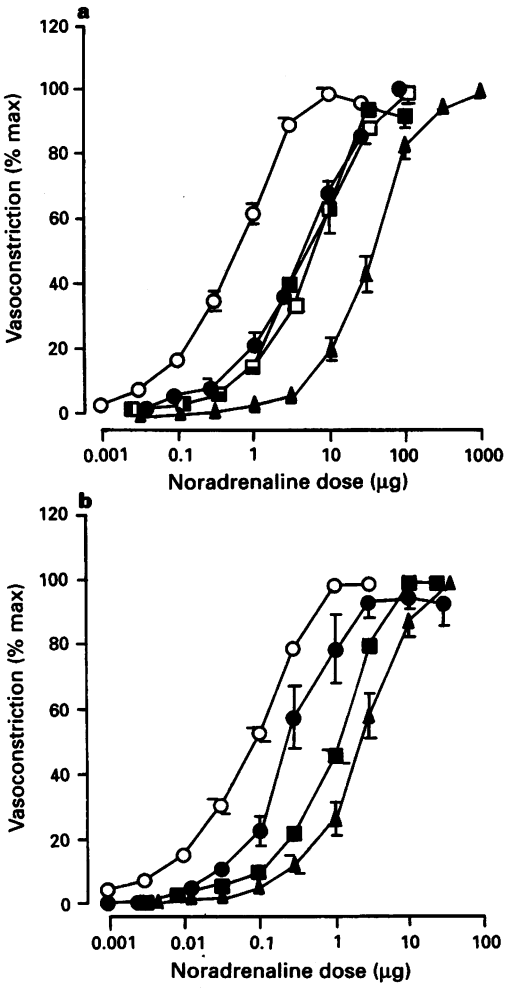

Figure 5 Effects of increasing concentrations of fenoldopam on the dose-response curves for vasoconstriction of the rat isolated perfused kidney by noradrenaline in the absence (a) and presence (b) of uptake blockade by cocaine and metanephrine. Curves were obtained in the absence (○) and repeated once in the presence of fenoldopam, 10^{-6} (□); 3×10^{-6} M (●); 10^{-5} M (■); 3×10^{-5} M (▲). Points are the mean responses expressed as a percentage of the maximum response (± s.e.mean, $n \geq 4$). Pre-antagonist curves are corrected from control experiments.

Region I, a fixed proportion of the agonist is removed from the vicinity of the receptor. As the agonist concentration is increased (for example, to overcome the effect of a competitive antagonist), an increasing proportion of the agonist reaches the receptor due to progressive saturation of the uptake mechanism (Region II) until saturation of uptake is virtually complete and the concentration of agonist at the receptor approaches that in the surrounding medium (Region III). As dose-response curves to the agonist are shifted progressively to the right by increases in antagonist concentration, they pass from Region I, through Region II, in which the increasing proportion of the agonist reaching the receptor has a potentiating effect causing steepening, and sinistral (leftwards) displacement of the EC_{50}. Thus, the dose-ratios are reduced and the slope of the Schild plot is reduced.

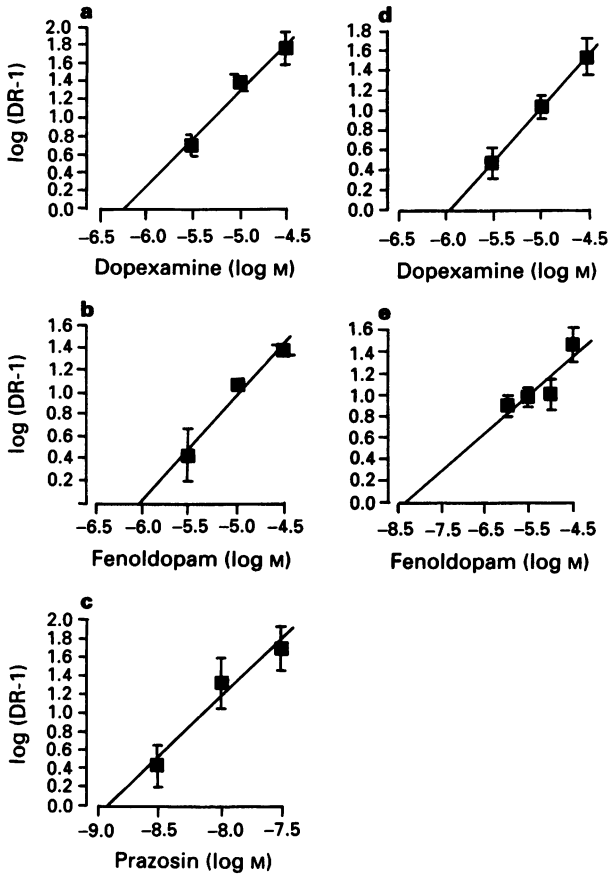

Figure 6 Schild plots for the antagonism of the vasoconstrictor effects of noradrenaline in the rat isolated perfused kidney by dopexamine, fenoldopam and prazosin. Experiments were performed either in the presence of uptake blockade by cocaine (10^{-5} M) and metanephrine (10^{-5} M) (dopexamine a, fenoldopam b, prazosin c) or in the absence of uptake blockade (dopexamine d, fenoldopam e). Points are mean values ± s.e.mean ($n \geq 4$).

Figure 6e shows that in the absence of uptake block, no increase in dose-ratio was observed as the concentration of fenoldopam was increased from 10^{-6} M to 10^{-5} M. Only when the concentration of fenoldopam was raised to 3×10^{-5} M did the dose-ratio appreciate. According to the Langer & Trendelenberg (1969) model, 3×10^{-6} M and 10^{-5} M fenoldopam may have shifted the noradrenaline dose-response curves into Region II, where an increasing proportion of the agonist reaches the receptor due to progressive saturation of neuronal uptake. The resulting potentiation of the noradrenaline responses was sufficient to nullify the effect of increasing the fenoldopam concentration such that the dose-response curves were shifted no further rightwards than in the presence of 10^{-6} M fenoldopam (Figure 5a). This argument assumes that although higher added concentrations of noradrenaline were not in fact used in the presence of 3×10^{-6} and 10^{-5} M fenoldopam, there may be a higher concentration at the receptor. When the antagonist concentration was increased to 3×10^{-5} M, the noradrenaline dose-response curves were shifted into Region III in which saturation of uptake is virtually complete, and with no further opposition to the action of fenoldopam possible, the noradrenaline dose-response curve was driven further rightwards with a consequent increase in ED_{50} and dose-ratio. Due to the magnitude of this final dose-ratio, the slope of the Schild plot was not drastically reduced by the failure of 3×10^{-6} M and 10^{-5} M fenoldopam to increase the dose-ratio appreciably from its value of 10^{-6} M. Rather, the major cause of the reduced slope was the large magnitude of the dose-ratios associated with the 10^{-6}–10^{-5} M concentrations of the antagonist. This, in turn, was the result of an effect associated with the correction (for time-dependent changes in sensitivity) of the pre-antagonist dose-response curve.

Figure 7 shows noradrenaline dose-response curves from time-matched control experiments. The curves were constructed before and after a 'sham' incubation period equivalent to the contact time with fenoldopam. Contrary to expectations, in the absence of uptake block (Figure 7a), Curve 2 was sinistrally displaced; if anything, a dextral displacement was anticipated due to the attenuation of tissue sensitivity with repeated exposure to noradrenaline. In the presence of uptake block, the two curves were virtually superimposed (Figure 7b), suggesting that the operation of the uptake mechanism was, paradoxically, responsible for potentiation of the responses to noradrenaline on repeated exposure.

One possible explanation for this is that supramaximal doses of noradrenaline required to confirm the maximum response may have persisted in the uptake mechanism during washout and resulted in potentiation of responses in the second curve. This is supported by the fact that the ED_{50} for the second curve constructed in the absence of uptake block (63 (37–109)ng) was not significantly different from the ED_{50} for the corresponding curve obtained in the presence of uptake block (56 (40–77)ng). The pre-antagonist curve was therefore displaced to the left by the correction procedure (Figure 8a) and this exaggerated the dose-ratios to a proportionally greater extent at lower concentrations of fenoldopam. This effect caused the marked reduction of the slope of the Schild plot in the absence of uptake block compared

Table 1 Values for pA_2 and Schild plot slope (± s.e.mean) for the antagonism of noradrenaline-induced vasoconstriction of the rat perfused kidney by fenoldopam, dopexamine and prazosin

	No uptake block		*Uptake blocked*		
	Dopexamine	*Fenoldopam*	*Dopexamine*	*Fenoldopam*	*Prazosin*
Slope	1.08 ±0.20	0.35† ±0.12	1.05 ±0.18	0.94 ±0.06	1.28* ±0.16
pA_2	5.95 ±0.21	8.36 ±0.21	6.23^{NS} ±0.11	6.02†† ±0.21	8.91 ±0.58

Uptake mechanisms were blocked with cocaine (10^{-5} M) and metanephrine (10^{-5} M) *Significantly greater than 1.0; †Significantly less than 1.0 by Student's t test ($P<0.05$), ††significantly different ($P<0.05$) or ^{NS}not significantly different from value with no uptake block.

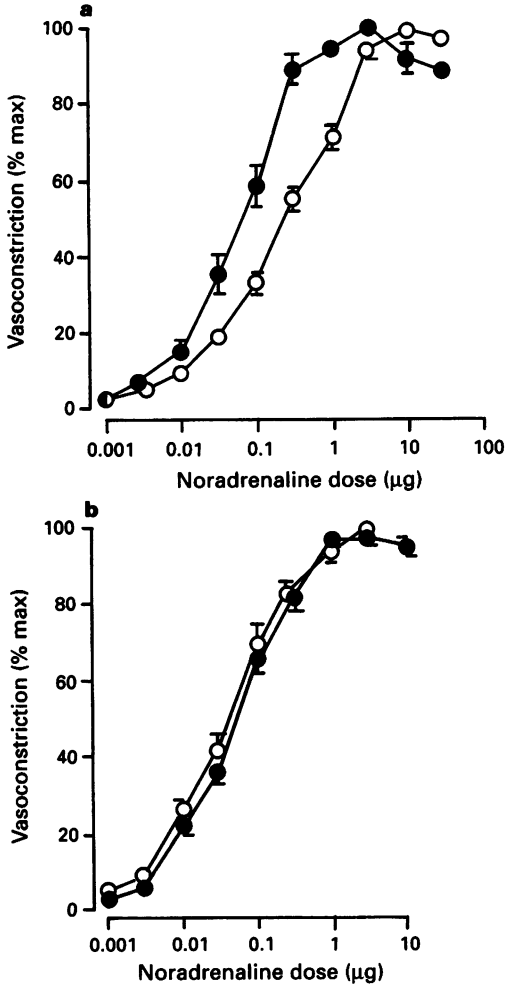

Figure 7 Dose-response curves for vasoconstrictor responses to noradrenaline from time-matched control experiments conducted in the absence of antagonist and obtained in the absence (a) or presence (b) of uptake block. The initial dose-response curve (○) was followed after a sham-incubation without antagonist by a second curve (●). Points are the mean responses expressed as a percentage of the maximum response (± s.e.mean, $n \geqslant 4$).

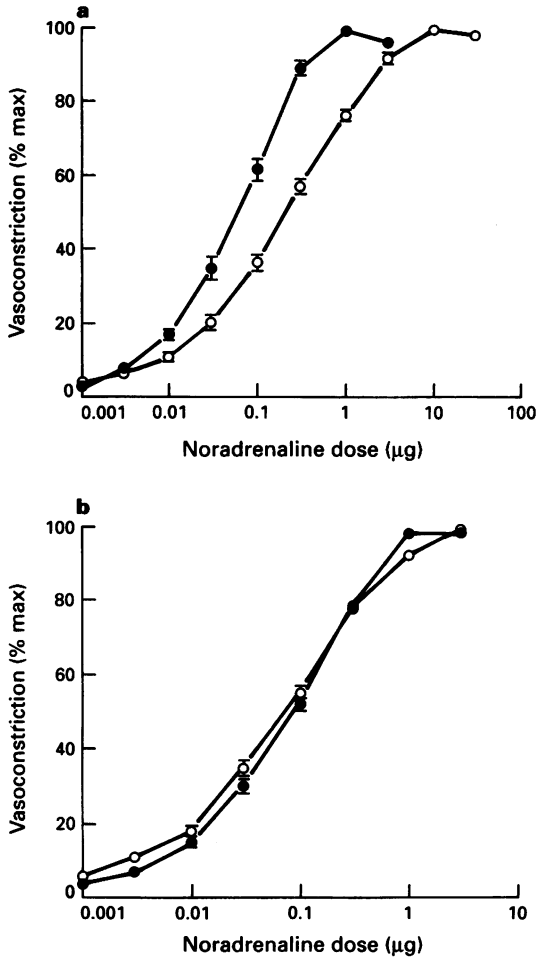

Figure 8 Effects of correction for time-dependent changes in tissue sensitivity upon the pre-antagonist (fenoldopam) noradrenaline dose-response curves. The pre-antagonist curves ($n \geqslant 12$) obtained in the absence (a) or presence (b) of uptake blockade with cocaine (10^{-5} M) and metanephrine (10^{-5} M) are shown. The uncorrected (○) curves and those after correction from control experiments (●) are shown. Points are the mean vasoconstrictor responses expressed as a percentage of maximum response (± s.e.mean).

with that obtained in the presence of uptake block. This indicates the importance of inhibiting the uptake mechanism when using noradrenaline as the agonist.

Effect of uptake block on antagonism of noradrenaline responses by dopexamine

Unlike fenoldopam, dopexamine progressively shifted the noradrenaline dose-response curves rightwards in a concentration-dependent manner in the absence as well as in the presence of uptake block (Figure 4a). This was reflected in the slopes of the Schild plots which were not significantly different from unity in either case. The most obvious reason for dopexamine producing a unity Schild plot slope in the absence of uptake block is that it is a more potent inhibitor of neuronal uptake than cocaine (Mitchell *et al.*, 1987; Nedergaard, 1988; 1989). Inhibition of uptake by dopexamine would potentiate dose-response curves to noradrenaline and thus lessen the degree of antagonism. According to the Langer & Trendelenburg model (1969), there would be no potentiation of the dose-response curve generated in the absence of the antagonist, and dose-ratios would consequently be reduced to a greater extent at low concentrations of dopexamine than at high concentrations. This would result in a steepening of the Schild plot to maintain unity slope in the absence of cocaine.

Effect of prazosin on the responses to noradrenaline

In the presence of uptake blockade, the antagonism of the vasoconstrictor responses of noradrenaline by prazosin resulted in a Schild plot having a slope which was significantly greater than unity. Schild plot slopes exceeding unity can result from lack of equilibrium conditions for the antagonist at the time the response is measured, or from loss of free drug from the external solution (Furchgott, 1972). In addition, a saturable removal mechanism for the antagonist can also give rise to high slopes (Kenakin, 1987). The latter cause can be discounted since prazosin is not a substrate for neuronal or extraneuronal uptake which were, in any case, blocked with cocaine and metanephrine respectively. It is possible that the time allowed for equilibration of prazosin with the receptor (30 min) was not sufficient. Since the rate of onset of antagonist action is concentration-dependent, the effect of low concentrations of prazosin with this length of equilibration may be underestimated, resulting in a steepening of the slope of the Schild plot (Kenakin, 1987). Indeed, in the present case, a disproportionately low dose-ratio value at the lowest concentration of prazosin was the main cause of the high slope obtained. Another possible explanation for the high slope of the Schild plot for prazosin is that bolus injections of noradrenaline, being short-lived in the system, do not allow sufficient time for the drug to reach equilibrium.

As in the present study, Blue & Clarke (1992) found that antagonism by prazosin of the vasoconstrictor responses produced by bolus doses of noradrenaline in the rat perfused kidney apparently deviated from competitive inhibition; the slope of the Schild plot obtained was significantly greater than unity (1.33). On the other hand, when noradrenaline was administered by infusion, the Schild plot for antagonism of the responses by prazosin was not significantly different from unity, indicating competitive kinetics. These authors proposed that lack of equilibrium conditions for the agonist, due to the inherent transience of bolus doses in a perfused system, can lead to a progressive overestimation of the dose-ratio of the antagonist and thus to the slope of the Schild plot exceeding unity when high affinity antagonists such as prazosin are employed. In the present study, the slope of the Schild plot for the antagonism of noradrenaline by prazosin was greater than unity whereas both fenoldopam and dopexamine (in the presence of uptake block) produced slopes which were not significantly different from unity. The hypothesis of Blue & Clarke (1992) can account for this difference in that fenoldopam and dopexamine are weaker antagonists ($pA_2 = 6.2$ and 6.0 respectively, see above) than prazosin, and as such their relatively rapid onset/offset rates would allow both noradrenaline and antagonist (dopexamine or fenoldopam) to attain equilibrium with the α_1-adrenoceptor in spite of the rapid removal of injected noradrenaline from the perfused kidney.

The finding that the two D_1-receptor agonists, dopexamine and fenoldopam, also possess antagonist activity at α_1-adrenoceptors confirmed previous observations for fenoldopam by Ohlstein *et al.* (1985). This property would probably contribute to the antihypertensive activity of fenoldopam (Ventura *et al.*, 1984). α_1-Adrenoceptor blockade by dopexamine would also complement its agonist activity at D_1-dopamine receptors and β_2-adrenoceptors by reducing sympathetic vasoconstrictor tone to the renal vasculature and improving renal blood flow. The reduced vascular tone would also exert favourable effects on cardiac pre- and afterload to improve cardiac performance in low output states. The fact that the vasodilator activities observed in the present study could be attributed to α_1-adrenoceptor blockade was dependent upon the use of noradrenaline to maintain vascular tone. It does not imply that these compounds cannot exert vasodilatation via D_1-receptors when an alternative vasoconstrictor is employed (Schmidt *et al.*, 1987).

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