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The effect of ketotifen on nitric oxide synthase activity

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1 We studied the effect of ketotifen, a second generation H_1-receptor antagonist on nitric oxide synthase (NOS) activity in colonic mucosa and in renal tissues, and on rat renal haemodynamics *in vivo*.

2 Ketotifen (100 μg ml^{-1}) increased human colonic NOS activity from 3.7 ± 0.6 to 14.5 ± 1.3 nmol g^{-1} min^{-1} ($P < 0.005$, ANOVA). In rat renal cortical and medullary tissues ketotifen increased NOS activity by 55% and 86%, respectively ($P < 0.001$). The stimulation of NOS activity was attenuated by NADPH deletion and by the addition of N^{ω} nitro-L-arginine methyl ester (L-NAME) or aminoguanidine, but not by [Ca^{2+}] deprivation. NOS activity was unaffected by two other H_1-antagonists, diphenhydramine and astemizole, or by the structurally related cyproheptadine. Renal cortical NOS activity was also significantly stimulated 90 min after intravenous administration of ketotifen to anaesthetized rats.

3 Ketotifen administration to anaesthetized rats induced modest declines in blood pressure and reduced total renal, cortical and outer medullary vascular resistance. This is in contrast to diphenhydramine, which did not induce renal vasodilatation.

4 We conclude that ketotifen stimulates NOS activity by mechanisms other than H_1-receptor antagonism. The association of this effect with therapeutic characteristics of ketotifen and the clinical implications of these findings are yet to be defined.

Keywords: Ketotifen; diphenhydramine; astemizole; kidney; colon; microcirculation; nitric oxide; nitric oxide synthase; haemodynamics

Introduction

Ketotifen, a second generation histamine H_1-receptor antagonist has long been used in the management of allergic disorders, including bronchial asthma and atopic dermatitis (Craps, 1984; Grant *et al*., 1990). Recently, ketotifen was found to reduce ileal damage induced by *Clostridium-difficile* toxin A (Pothoulakis *et al*., 1993) and to attenuate experimental colitis (Eliakim *et al*., 1992; 1995). It was also found to prevent gastric mucosal damage from ethanol, indomethacin and hydrochloric acid in rats (Karmeli *et al*., 1991) and to protect gastric mucosa from damage induced by non-steroidal anti-inflammatory drugs in healthy volunteers (Eliakim *et al*., 1993).

In addition to histamine-receptor antagonism, some of these effects may be related to the inhibition of the release of mast-cell and neutrophil-derived pro-inflammatory mediators. In various experimental and clinical conditions ketotifen was noted to reduce mast cell degranulation and to decrease the release of histamine, mast-cell proteases, myeloperoxidase, leukotrienes, platelet-activating factor (PAF) and various prostaglandins (Craps, 1984; Karmeli *et al*., 1991; Pothoulakis *et al*., 1993; Gronbech & Lacy, 1994). Ketotifen also inhibits polymorphonuclear aggregation and migration, and attenuates inflammatory responses (Pothoulakis *et al*., 1993). It also directly reduces eosinophil function (Nabe *et al*., 1991) and viability (Hossain *et al*., 1994).

Since nitric oxide (NO) inhibits mast cell pro-inflammatory reactions (Kubes *et al*., 1993; Hogaboam *et al*., 1993; Kanwar *et al*., 1994; Masini *et al*., 1994; Suematsu *et al*., 1994) and has profound effects on gastric and intestinal mucosa (Salzman, 1995), we studied the effect of ketotifen on NO synthesis. In this work we show that inducible nitric oxide synthase (NOS) activity is enhanced *in vitro* by ketotifen, but not by other H_1-receptor antagonists or by the structurally related cyproheptadine. We also studied renal NOS activity following ketotifen administration *in vivo* and evaluated its effects on renal haemoperfusion, which largely depends on nitro-vasodilatation (Agmon *et al*., 1994).

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Methods

Determination of NOS activity

NOS activity was determined by monitoring the conversion of [^{3}H]-L-arginine to citrulline (Bush *et al*., 1992). Tissue samples (10–15 mg wet weight) were homogenized for 30 s at 4°C with a Polytron (Kinematica, GmbH, Krienz-Luzerne, Switzerland) with 9 volumes of ice-cold 50 mM Tris HCl, pH 7.4, containing 0.1 mM EDTA, 0.1 mM EGTA, 0.5 mM dithiothreitol and 1 mM phenylmethylsulphonyl fluoride. Homogenates were centrifuged at 20,000 *g* for 60 min at 4°C and the supernatant was used as the source of nitric oxide synthase. Enzymatic reactions were conducted at 37°C in 50 mM Tris HCl, pH 7.4, containing 100 μM nicotine-amine adenine dinucleotide phosphate, reduced form (NADPH), 2 mM $CaCl_2$, 0.20–0.40 mg supernatant proteins and about 200,000 d.p.m. of L-[2,3,4,5-^{3}H]-arginine HCl (77 Ci mmol^{-1}) (Amersham) to a final volume of 100 μl. Enzymatic reactions were terminated by the addition of 2 ml of ice cold 'stop buffer' containing 20 mM sodium acetate, pH 5.5, 1 mM L-citrulline, 2 mM EDTA and 0.2 mM EGTA.

Citrulline was determined by applying the samples (2 ml), prepared as described above, to columns (1 cm diameter) containing 1 ml of Dowex AG50W-X8, Na form which had been pre-equilibrated with stop buffer. Columns were eluted with 4×1.0 ml of water and collected into scintillation vials. Opti-Flour (10 ml) was added to each vial and samples were counted in a Packard liquid scintillation spectrometer. Citrulline was recovered in the first 4.0 ml of the Dowex column eluate to the extent of $96 \pm 2\%$. Enzyme activities performed in duplicate showed variability of 2%.

Manipulations of NOS activity NOS activity was determined with or without the addition of ketotifen (1–100 μg ml^{-1}), other antihistamines and cyproheptadine. As detailed below, complementary experiments were performed in incubation medium in which [Ca^{2+}] was deleted (with EGTA 1 mM and without $CaCl_2$) for selective inhibition of the constitutive isoform of NOS, or NADPH for non-selective inhibition of NOS, or with the addition of aminoguanidine (10 mM) for predominant inhibition of the inducible isoform, or N$^{\omega}$ nitro-L-arginine methyl ester HCl (L-NAME) (1 to 100 mM), a non-selective inhibitor (Fukuto & Chaudhuri, 1995).

Specificity of the assay to determine NOS activity This was also tested by the addition of valine (60 mM), which blocks arginase, in order to confirm that all citrulline recovered was a product of the NOS pathway.

In gastric and colonic mucosa we consistently found a linear correlation between tissue sample weight and protein content (gram wet weight = 100 mg protein, $r = 0.9$, $n = 27$, $P < 0.0001$). For this reason protein content in tissue samples was not determined and results of NOS activity are presented as the amount of labelled citrulline generation (nmol min^{-1} g^{-1} wet weight).

Effect of ketotifen on cytosolic NOS activity in vitro

Experiments were undertaken in human colonic mucosa and in rat renal tissues.

Studies in normal colonic mucosa With the approval of the institution's Helsinki Committee, human colonic mucosa was collected from tumour-free margins of resected colons. Samples were obtained from fresh surgical specimens and stored at −70°C until determination of NOS activity.

For dose-response relationships samples were incubated for 30 min with ketotifen at final concentrations of 0, 1, 10 and 100 μg ml^{-1}. The 100 μg ml^{-1} concentration was chosen for all further studies because it provided the best response.

According to time-response curves performed with tissue homogenates, incubated without (control) or with ketotifen, and with or without NADPH, a 30 min incubation period was chosen for all further studies in colonic mucosa.

The effects of ketotifen, L-NAME, aminoguanidine and valine were evaluated in pairs of mucosal specimens simultaneously incubated without (control) and with ketotifen. Additional samples with or without ketotifen were incubated with L-NAME (10 and 100 mM), aminoguanidine (10 mM) or valine (60 mM) and during removal of calcium.

Studies in rat renal tissues Kidneys were rapidly removed from 5 anaesthetized, intact rats (Inactin, BYK Gulden, Konstanz, Germany, 100 mg kg^{-1} body weight) through mid-abdominal incision and dissected on ice. Cortex and medulla (including outer medulla and papilla) were separated and stored at −70°C. According to a time-response curve, performed as detailed above, the incubation period for renal tissues was set at 15 min. Cortical and medullary cytosolic NOS activity was determined without (control) and with ketotifen, in complete reaction mixture, with deletion of [Ca^{2+}] or NADPH, or with the addition of L-NAME (1 mM).

In order to explore the role of H_1-receptor antagonism in NOS stimulation, the effects of diphenhydramine (first generation H_1-antagonist, 100 μg ml^{-1}) and astemizole (second generation drug, 100 μg ml^{-1}) were also studied (Simons & Simons, 1994). Since therapeutic doses of ketotifen, astemizole and diphenhydramine are in the range of 1, 10 and 100 mg day^{-1}, respectively, additional studies were performed with the latter two agents at concentrations of 1 and 10 mg ml^{-1}. The effect of cyproheptadine (100 μg ml^{-1}), which structurally resembles ketotifen, was also evaluated.

Effect of ketotifen on renal cytosolic NOS activity in vivo

Fourteen Sprague-Dawley rats (260–440 g weight) fed on regular rat chow and water *ad libitum* were used. Under anaesthesia (pentobarbitone 50 mg kg^{-1}) PE50 catheters were inserted into the right femoral vein and artery for drug administration and blood sampling, respectively. Rats were injected intravenously with either saline (control rats) or ketotifen (1 mg kg^{-1}, dissolved in 0.9% NaCl at a concentration of 1 mg ml^{-1}). Ninety minutes later the rats were killed and the kidneys rapidly removed and processed as detailed above.

NOS was determined separately in the cortex and medulla of control and ketotifen-treated rats. In addition, these tissues were concomitantly incubated in medium in which [Ca^{2+}] or NADPH were absent, or with the addition of L-NAME (1 mM).

Effect of ketotifen on renal microcirculation

In order to evaluate the possible effects of NOS stimulation by ketotifen on haemodynamic and microcirculatory parameters, changes in mean blood pressure and total and regional renal blood flows were studied in male Sprague-Dawley rats (315–535 g weight), anaesthetized with Inactin. The renal vasculature was chosen for evaluation since nitro-vasodilatation participates in its haemodynamic regulation under basal conditions and in response to vasoconstrictive stimuli (Tolins *et al*., 1990; Majid & Navar, 1992; Agmon *et al*., 1994). As previously detailed (Agmon *et al*., 1994; Heyman *et al*., 1995), tracheotomy was performed and the right femoral vein and artery were cannulated with PE50 catheters (Clay-Adams, Parsippany, NJ) for the infusion of normal saline (0.11 ml min^{-1}) and for blood pressure monitoring. The left kidney was exposed by a mid-abdominal incision and mechanically fixed. The temperature of the kidney was monitored by a needle copper probe connected to a type T thermocouple (Omega Engineering, Stamford, CT) and maintained at 38°C with a heating lamp and by dripping warm saline and mineral oil.

Mean blood pressure was monitored by a calibrated pressure transducer and monitor (Hewlett Packard Inc., Sunnyvale, CA) connected to the femoral arterial line. Renal blood flow was monitored by a perivascular transonic ultrasonic volume flow sensor (T-106, Transonic Systems Inc., Ithaca, NY) with the probe mounted on the left renal artery.

Selective regional blood flow was measured by laser Doppler probes connected to flowmeters (models PF2B and PF3,

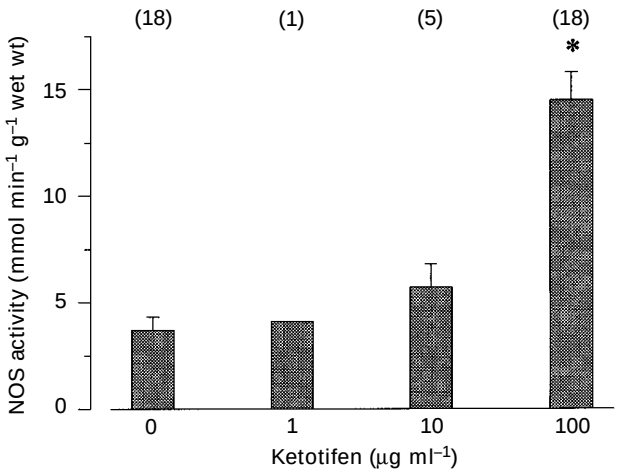

Figure 1 Effect of different doses of ketotifen on NOS activity in human colonic mucosa *in vitro*. NOS activity is expressed as citrulline generation (nmol min^{-1} g^{-1} wet wt.). Results are mean ± s.e. of number of experiments shown in parentheses.

Perimed, Stockholm, Sweden). A superficial probe (1 mm diameter) monitored cortical blood flow while a needle probe (0.45 mm diameter), inserted at a depth of 4–4.5 mm, recorded outer medullary blood flow. Its proper position within the outer medulla was confirmed at the conclusion of the experiment.

Table 1 Effect of ketotifen on human colonic NOS activity

Incubation Medium	*Regular* (18)	*+Valine* (6)	*−[Ca^{2+}]* (4)	*+Amino-guanidine* (3)	*+L-NAME 10 mM* (6)	*+L-NAME 100 mM* (3)
Control	3.7±0.6	2.8±1.8	5.8±2.2	1.0±0.5[b]	1.2±0.3	0.3±0.2[b]
Ketotifen	14.5±1.3[a]	12.6±2.3[a]	11.7±3.3[a]	6.2±2.3[c]	13.5±2.0[a]	6.0±1.0[c]

Tissue homogenates were incubated for 30 min without (control) and with ketotifen (100 $\mu g\,ml^{-1}$), in regular medium, detailed in Methods, in medium deprived of calcium, or with the addition of valine (60 mM), aminoguanidine (10 mM) or L-NAME (10 and 100 mM). NOS activity is expressed in nmol min^{-1} g^{-1} wet wt. Results are mean±s.e. of number of experiments shown in parentheses. [a]$P<0.01$ vs control at regular medium (ANOVA). [b]$P<0.05$ vs control at regular medium (by t test, only). [c]$P<0.01$ vs ketotifen at regular medium (ANOVA).

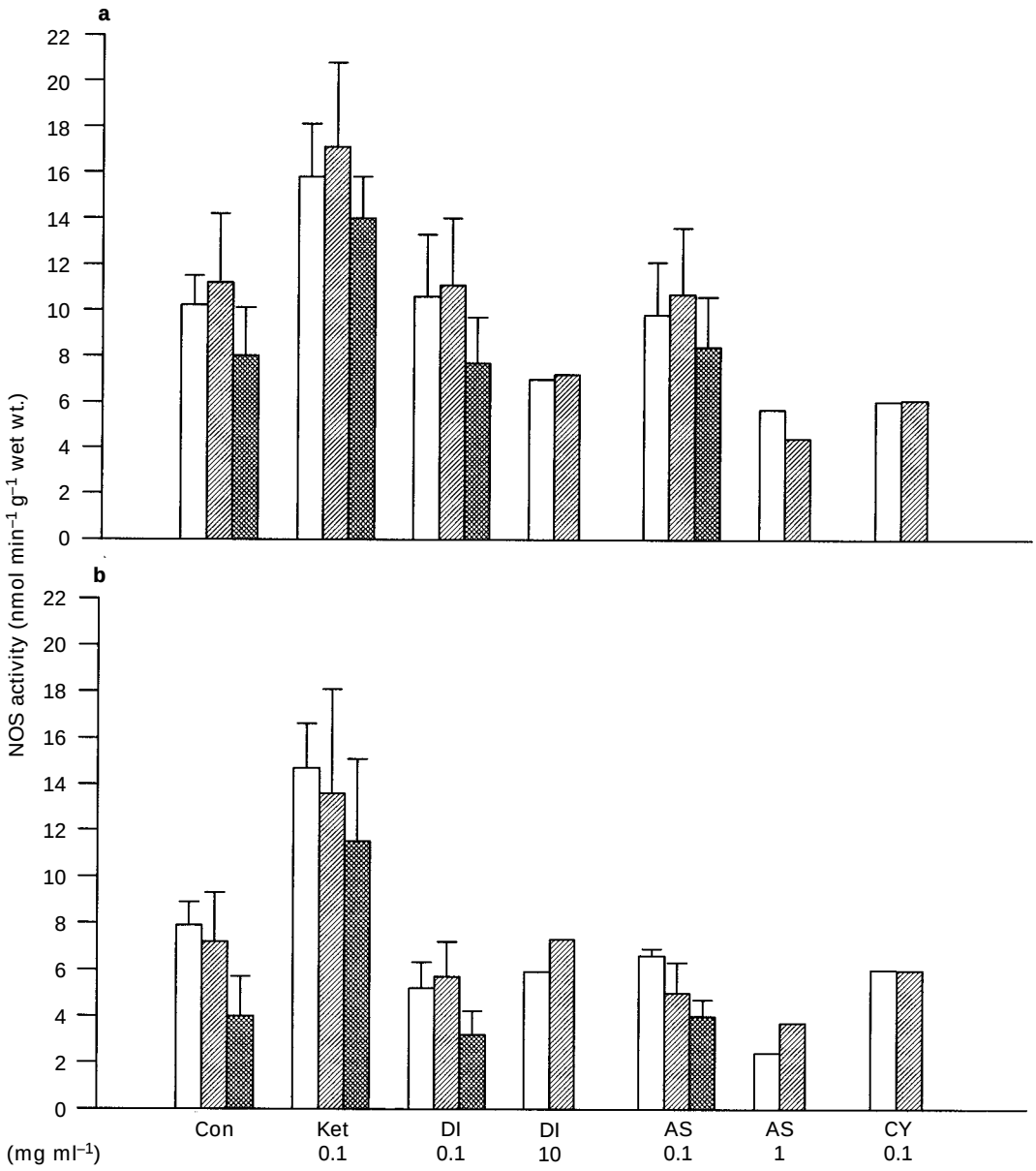

Figure 2 Effect of ketotifen on NOS activity *in vitro* in the rat renal cortex (a) and medulla (b). Tissue homogenates were incubated without (control-con) and with ketotifen (Ket) (100 $\mu g\ ml^{-1}$), diphenhydramine (DI) (100 $\mu g\ ml^{-1}$, 10 mg ml^{-1}), astemizole (AS) (100 $\mu g\ ml^{-1}$, 1 mg ml^{-1}), or cyproheptadine (CY) (100 $\mu g\ ml^{-1}$) in the presence (open columns) and absence (hatched columns) of [Ca^{2+}], or with the addition of L-NAME (1 mM) (cross-hatched columns). NOS activity in the ketotifen treatment is greater than all other groups, while L-NAME inhibits NOS activity ($P<0.001$, two-way ANOVA). ($n=3-7$ for each column with an error bar, or $n=1$ otherwise). NOS activity is expressed as citrulline generation (nmol min^{-1} g^{-1} wet wt.).

Changes in mean blood pressure, total renal and selective cortical and medullary blood flows were recorded before and after the injection of ketotifen or diphenhydramine (six and four rats, respectively). Following 30 min of stabilization and baseline recordings, ketotifen or diphenhydramine were administered intravenously at increasing doses (0.1 mg kg^{-1}, 0.5 mg kg^{-1} and 1 mg kg^{-1}) at 15 min intervals.

On-line recordings were displayed, stored and analysed with a computerized system (MacLab/8, World Precision Instruments, Inc., Sarasota, FL). Mean values were determined over 10 min periods before (baseline) and 5 min after each dose of ketotifen. Changes in total and regional renal vascular resistance were calculated from the ratio of blood pressure to flow. Data are expressed as the percentage change from baseline values.

The various H_1-antagonists, as well as cyproheptadine, aminoguanidine, valine and L-NAME were purchased from Sigma Chemical Co. (St. Louis, MO).

Statistics

Values are presented as the means ± s.e.mean. One-way analysis of variance was applied for comparisons of repeated measurements in both sets of *in vivo* studies. Two-way analysis of variance was carried out in the evaluation of differences between H_1-receptor antagonists at the various incubation media. Newman-Keuls test was used for *post hoc* multiple comparisons. Student's *t* test was applied for all other comparisons and statistical significance was set at $P<0.05$.

Results

Effect of ketotifen on cytosolic NOS activity in vitro

Experiments in colonic mucosa Ketotifen, at a concentration of 100 μg ml^{-1}, stimulated NOS activity while 1 and 10 μg ml^{-1} had no significant effect (Figure 1). All further experiments were carried out at concentration of 100 μg ml^{-1}. Colonic NOS activity, incubated with ketotifen, was 14.5 ± 1.3 ($n=18$) versus 3.7 ± 0.6 nmol min^{-1} g^{-1} wet wt. in paired controls ($P<0.005$). Colonic NOS activity was not affected by valine or calcium depletion but was inhibited by aminoguanidine (Table 1). L-NAME (100 mM) induced a 50% inhibition of ketotifen-stimulated NOS activity by comparison with 92% inhibition of NOS activity in controls (Table 1).

Experiments in renal tissues Figure 2 shows a summary of the experiments in the cortex (a) and medulla (b), indicating that in both tissue homogenates ketotifen (100 μg ml^{-1}) augmented NOS activity by 55% and 86%, respectively ($P<0.001$, ANOVA). Ketotifen-stimulated renal NOS activity was not affected by $[Ca^{2+}]$ depletion but was attenuated by L-NAME. NOS activity in both cortex and medulla was unaffected by diphenhydramine and astemizole (100 μg ml^{-1}). These agents, at concentrations 10 and 100 times higher, respectively, as well as cyproheptadine (100 μg ml^{-1}) also failed to enhance NOS activity.

Effect of ketotifen on renal NOS in vivo

Ninety minutes after the administration of saline or ketotifen (1 mg kg^{-1}) to anaesthetized rats NOS activity in the renal cortex was 55% higher in ketotifen-treated animals, as compared with controls ($P<0.005$) (Table 2). Medullary NOS activity was comparable in the two experimental groups. As in the studies *in vitro*, NOS activity in renal tissues in both control and ketotifen treated rats was not affected by $[Ca^{2+}]$ deprivation. Inhibition of cortical NOS by NADPH deprivation or L-NAME was comparable in ketotifen-treated and control rats.

Effects of ketotifen on the renal microcirculation

The repeated intravenous administration of rising doses of ketotifen resulted in a fall in mean blood pressure and some rise in renal blood flows over 45 min (Figure 3a and b). Consistent and significant falls in total, cortical and medullary renal vascular resistance were noted, most prominent at the cortical level. In contrast, the haemodynamic response to diphenhydramine, administered in the same manner, was unremarkable (Figure 3c and d).

Discussion

Nitric oxide (NO) is produced from L-arginine by the two isoforms of the enzyme, nitric oxide synthase (NOS) (the constitutive isoform (cNOS) which is calcium-dependent; and the inducible isoform (iNOS) which is calcium- and calmodulin-independent) and is activated by agents, such as lipopolysaccharide (LPS) and cytokines (Moncada & Higgs, 1993; Lowenstein *et al*., 1994). Tissues and cells differ with respect to the different expressions of the two isoforms. Whereas the inducible isoform is the mainly expressed in inflammatory cells, both isoforms are present in gastrointestinal mucosa (Nichols *et al*., 1993; Salzman, 1995) and renal tissues (Terada *et al*., 1992; Morrissey *et al*., 1994). Small amounts of NO generated mainly by the constitutive isoform in endothelial cells are apparently essential in maintaining vasodilatation and tissue integrity. However, when generated in large quantities by the inducible isoform NO has been proposed as a mediator of bactericidal, tumourostatic and tumourocidal activity of macrophages (Moncada & Higgs, 1993; Lowenstein *et al*., 1994). Our inability to suppress basal NOS activity in all tissues examined by the calcium chelator EGTA suggests an overwhelming majority (?) of the inducible isoform.

So far, stimulation of NOS activity has been shown to be induced only by cytokines and LPS and only with respect to

Table 2 Effect of ketotifen on renal NOS activity *in vivo*

	Incubation medium	*Regular*	*−[Ca²⁺]*	*−NADPH*	*+L-NAME*
Cortex	Control	7.1 ± 0.2 ($n=6$)	7.3 ± 0.5 ($n=3$)	3.4 ± 1.8[a] ($n=3$)	5.3 ± 0.4[a] ($n=6$)
	Ketotifen	11.0 ± 0.7[b] ($n=8$)	8.2 ± 1.6 ($n=3$)	4.3 ± 1.1[a] ($n=3$)	7.1 ± 1.7[a] ($n=7$)
Medulla	Control	3.4 ± 0.4 ($n=3$)	ND	ND	ND
	Ketotifen	3.3 ± 0.6 ($n=4$)	ND	ND	ND

Kidneys were removed from anaesthetized rats 90 min following the injection of saline (controls) or ketotifen (1 mg kg^{-1}). NOS activity was determined in the cortex and medulla, incubated for 15 min in regular medium, in medium deprived of $[Ca^{2+}]$ or NADPH, or with the addition of L-NAME (1 mM). NOS activity is expressed in nmol min^{-1} g^{-1} wet wt. Results are mean ± s.e. of *n* experiments (ND = not determined). [a]$P<0.05$ vs regular medium (ANOVA). [b]$P<0.01$ vs control (Student's *t* test).

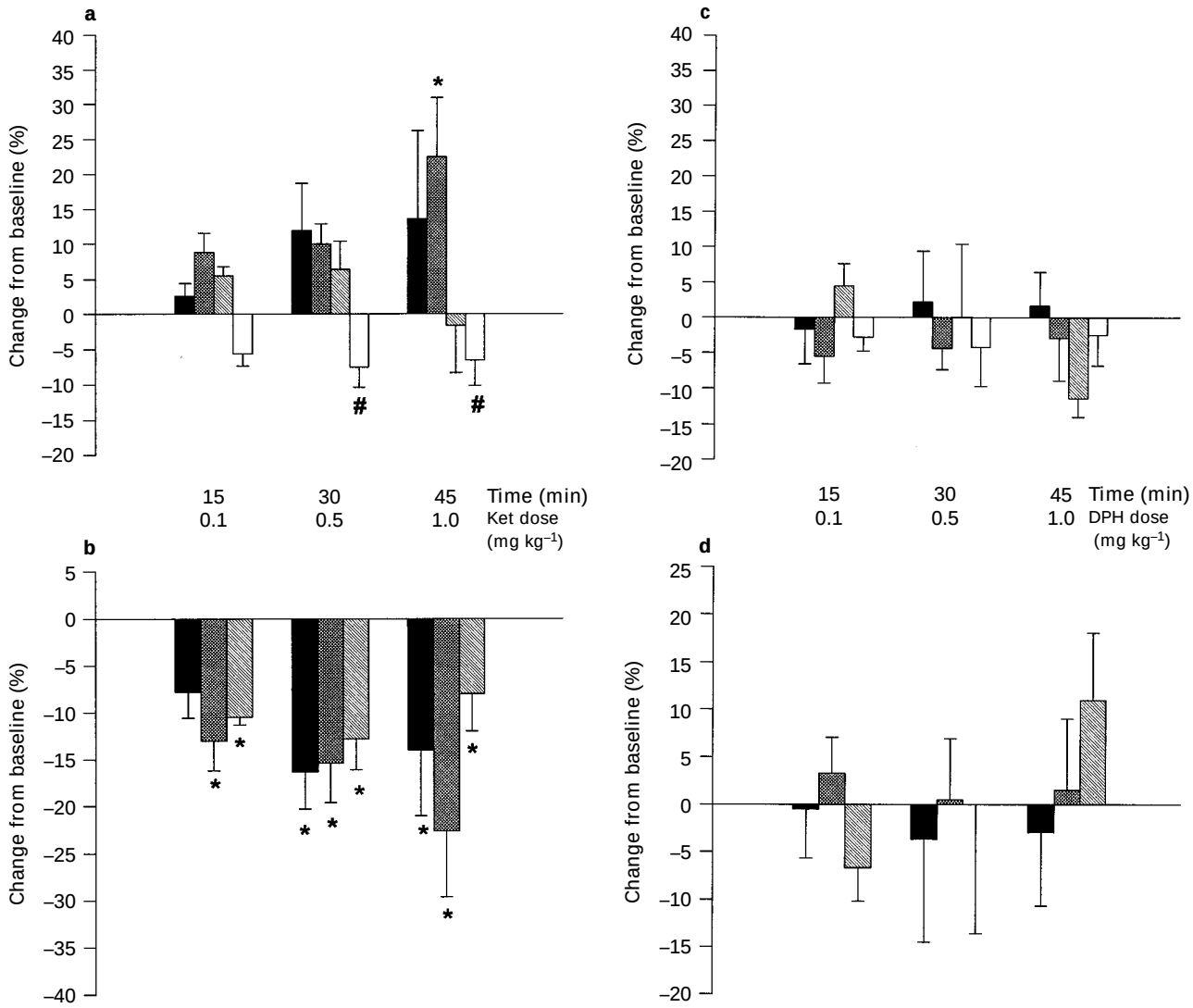

Figure 3 Changes in blood pressure, total and regional blood flows and in calculated renal vascular resistance of rats repeatedly injected over 45 min with increasing doses of (a and b) ketotifen (Ket, $n=6$) or (c and d) diphenhydramine (DPH, $n=4$). Note the vasodilator effects of ketotifen, which are absent following diphenhydramine. In (a and c) (solid columns) renal blood flow; (cross-hatched columns) cortical blood flow; (hatched columns) medullary blood flow; (open columns) blood pressure. In (b and d), (solid columns) total renal vascular resistance; (cross-hatched columns) cortical vascular resistance; (hatched columns) medullary vascular resistance. Results are mean ± s.e. ($^{*}P<0.05$ vs baseline, ANOVA; $^{\#}P<0.05$ vs baseline by paired t test, only).

the inducible isoform. In the present study, for the first time, pharmacological stimulation of NOS is shown to be induced by ketotifen. Since calcium deprivation does not attenuate NOS stimulation, it seems that ketotifen activates the inducible isoform. Activation of arginase by ketotifen was excluded because of its lack of effect of valine upon enzyme activity, thus confirming that in the enzymatic assay used, citrulline generation reflects NOS activity rather than arginase activity. In this series of experiments ketotifen was shown to stimulate, both *in vivo* and *in vitro*, the activity of the inducible isoform in the rat renal cortex and medulla, as well as in human colonic mucosa. A word of caution is warranted when applying ketotifen-related stimulation to the inducible isoform. The lack of effect of calcium deprivation in all our experiments, including basal conditions, raises the possibility of some background contamination with endotoxin which could dampen changes in cNOS, excess endogenous calcium or an unidentified interference with the dissociation of the cNOS-calmodulin complex. Nevertheless, the marked NOS inhibition by aminoguanidine (Table 1) strongly supports our suggestion of the predominance of iNOS, both under basal conditions and following ketotifen stimulation, since the latter isoform may be selectively inhibited by this agent (Griffiths *et al*., 1993; Misko *et al*., 1993). The non-specific inhibition of NOS by flavoprotein depletion (-NADPH) was also tested and found to be effective in the rat kidney (Table 2), as was the effect of the arginine analogue, L-NAME, both in human colonic mucosa (Table 1) and the rat kidney (Table 2 and Figure 2). The different magnitude of NOS inhibition by L-NAME, ranging from some 25% in the kidney to 60–90% in colonic mucosa probably results from the different concentrations of L-NAME, and may also reflect diverse responses of the various isoforms in different tissues to the arginine analogue (Fukuto & Chaudhuri, 1995).

Currently the major tools in the evaluation of the physiological and pathophysiological role of NO are the inhibition of NOS, supplementation of NOS substrate, L-arginine or the administration of other nitro-vasodilators. Pharmacological activation of NOS by ketotifen may turn out to be an additional experimental probe.

The clinical and therapeutic implications of NOS activation by ketotifen are yet to be defined. Defective nitro-vasodilatation has been implicated in association with hypertension, hyperlipidaemia, atherosclerosis, diabetes mellitus (Lerman & Burnett, 1992; Wang *et al*., 1993), ageing (Reckelhoff *et al*., 1994) and radiocontrast administration (Schwartz *et al*., 1994). Nitrovasodilatation also plays a critical role in the preservation of regional microcirculation during vasoconstrictive sti-

muli: its inhibition markedly enhances hypoxic renal outer medullary damage following the administration of radiocontrast agents and non-steroidal anti-inflammatory drugs (Brezis *et al*., 1991; Agmon *et al*., 1994). Thus, enhancement of nitro-vasodilatation by the addition of the enzyme substrate, L-arginine (Schwartz *et al*., 1994), or by the induction of NOS may have future therapeutic implications.

The haemodynamic responses to ketotifen, presented in Figure 3a, include relaxation of the renal cortical and medullary vasculature and reduction of blood pressure. Since iNOS is widely distributed in the rat kidney, predominantly in the outer medulla and in cortical glomeruli (Morrissey *et al*., 1994), the renal microcirculatory response to ketotifen might be mediated, at least in part, by nitro-vasodilatation. The absence of a haemodynamic response to diphenhydramine (Figure 3b), as well as a tendency for smaller hypertensive responses to L-NAME after treatment with ketotifen (data not shown), may support this hypothesis. However, further studies may be required to establish a cause and effect relationship between NOS induction and ketotifen-induced renal vasodilatation, since other vasoactive mediators may also be involved.

NOS activation may contribute to the protective effect of ketotifen upon gastric (Karmeli *et al*., 1991; Eliakim *et al*., 1993) and colonic mucosa (Eliakim *et al*., 1992). At low concentrations nitric oxide protects gastric epithelium (Gronbech & Lacy, 1994; Masuda *et al*., 1995; Takeuchi *et al*., 1995) and has a role in maintaining the integrity of the gastrointestinal tract (Salzman, 1995). On the other hand, the inducible isoform of NOS, by the elaboration of high, toxic amounts of nitric oxide, seems to participate in the tissue inflammatory response and mucosal damage (Salzman, 1995; Salzman *et al*., 1995). Intestinal inflammation is associated with stimulated NOS activity and with increased release of nitrites/nitrates (Karmeli *et al*., 1994), and NOS inhibition ameliorates chronic ileitis (Miller & Sadowska-Krowicka, 1993) and colitis (Rachmilewitz *et al*., 1995). Therefore, the role of NOS activation in the beneficial gastrointestinal effects of ketotifen requires further evaluation.

Ketotifen is currently used for the treatment of bronchial asthma. Though the rate of pulmonary nitric oxide production correlates with local inflammatory reaction (Hamid *et al*., 1993; Barnes & Liew, 1995), exogenous inhaled NO ameliorates bronchoconstriction (Hogman *et al*., 1993). NOS activation may, therefore, contribute to the therapeutic effect of ketotifen in asthmatic patients.

Finally, recent studies identified NO as an important regulator of mast cells and inflammation: inhibition of NOS results in activation of mast cells, increased epithelial permeability and leukocyte adhesion, while induction of endogenous production of NO attenuates these effects (Kubes *et al*., 1993; Hogaboam *et al*., 1993; Kanwar *et al*., 1994; Masini *et al*., 1994; Suematsu *et al*., 1994). Consequently, our findings that ketotifen activates NOS suggest that its anti-allergic properties may be partially mediated through the formation of NO.

The mechanism whereby ketotifen induces its effects on NOS activity is obscure. It is not related to H_1-receptor inhibition, in view of the observation that two other H_1-antagonists, diphenhydramine and astemizole, were shown to have no effect on the enzyme activity. The structurally related cyproheptadine also failed to modulate NOS activity. Noteworthy, the concentration of ketotifen required for NOS induction in our experiments was one to three orders of magnitude higher than the dose employed in clinical practice. Additional experiments addressing drug uptake and binding in the various tissues and cellular organelles may shed light upon this discrepancy. The cause and effect relationship between NOS induction by ketotifen and the drug actions on the various physiological systems should also be determined.

In conclusion, ketotifen is the first medication found to enhance NOS activity. This effect is mediated by mechanisms other than H_1-receptor antagonism. Our findings suggesting stimulation of the inducible isoform warrant further confirmation. The association of NOS activation with therapeutic characteristics of ketotifen and the clinical implications of these findings are yet to be defined.

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