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Clonidine-induced increase in osmolar clearance and free water clearance via activation of two distinct α_2-adrenoceptor sites

H.D. Intengan & [1,,†]D.D. Smyth

Departments of *Pharmacology & Therapeutics and †Internal Medicine, University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Manitoba, Canada, R3E OW3

1 Clonidine, an α_2-adrenoceptor agonist, will increase urine flow rate in the anaesthetized rat by increasing both free water and osmolar clearance. In the present study, we investigated whether these effects of clonidine were mediated at two sites which could be distinguished pharmacologically in uninephrectomized male Sprague-Dawley rats.

2 Clonidine (1.0 nmol kg^{-1} min^{-1}) infused into the renal artery increased osmolar and free water clearance. Following pretreatment with prazosin (0.15 mg kg^{-1}, i.v.), an antagonist with reported selectivity for the α_{2b}-adrenoceptor subtype, the increase in free water but not osmolar clearance was decreased. Pretreatment with the opioid receptor antagonist, naltrexone (3.0 mg kg^{-1}, i.v.) attenuated the increase in osmolar but not free water clearance. This disparate antagonism of clonidine by prazosin and naltrexone was consistent with two distinct sites.

3 We submit the hypothesis that the α_{2a}- and α_{2b}-adrenoceptor subtypes mediated the clonidine-induced osmolar and free water clearance. The blockade in free water clearance by prazosin indicated a possible role of the α_{2b}-adrenoceptor subtype whereas the α_{2a}-adrenoceptor subtype was considered as the site mediating the clonidine-induced increase in osmolar clearance. UK-14,304 (1.0 nmol kg^{-1} min^{-1}), a mixed α_2-adrenoceptor/imidazoline receptor agonist with selectivity for the α_{2a}-subtype, increased only osmolar clearance. This increase was blocked by naltrexone but not prazosin pretreatment. The imidazoline receptor was not involved, as naltrexone failed to alter the moxonidine (3.0 nmol kg^{-1} - min^{-1}) induced increase in osmolar clearance. These data suggested to us that the α_{2a}-/α_{2b}-subtype hypothesis should be investigated more closely in future studies.

4 These findings indicate that the increase in osmolar and free water clearance following clonidine can be distinguished pharmacologically indicating that two sites were involved. Furthermore, we propose the hypothesis that the α_{2a}-adrenoceptor subtype mediated osmolar clearance whereas the α_{2b}-subtype mediated free water clearance. The prazosin-sensitive increase in free water clearance following clonidine suggested a possible role for the α_{2b}-subtype. The naltrexone-sensitive increase in osmolar clearance following clonidine and UK-14,304 (but not moxonidine) suggested a possible role of the α_{2a}-subtype. Clearly, this postulate requires further study.

Keywords: Clonidine; moxonidine; UK-14,304; α_{2a}-adrenoceptor; α_{2b}-adrenoceptor; urine flow; sodium excretion

Introduction

The administration of α_2-adrenoceptor agonists *in vivo* will increase urine flow rate by increasing osmolar and free water clearance (Strandhoy *et al.*, 1982; Gellai & Ruffulo, 1987; Stanton *et al.*, 1987; Blandford & Smyth, 1988; 1990). At least two of these studies utilizing the α_2-adrenoceptor agonists, guanabenz and clonidine, suggested that these renal effects were mediated by two distinct sites (Strandhoy *et al.*, 1982; Blandford & Smyth, 1988; 1990). Consistent with this, preliminary studies in our laboratory indicated that these two putative sites could be dissociated pharmacologically (see below).

It has been proposed that opioids are involved in the renal response to clonidine (Pan & Gutkowska, 1988). We therefore determined the effects of naltrexone (non-selective opioid receptor antagonist) on the renal actions of clonidine. These preliminary experiments indicated that naltrexone selectively blocked the increase in osmolar clearance to clonidine while having no effect on the increase in free water clearance. We were also investigating the preliminary observation that prazosin (α_1-adrenoceptor antagonist with relative selectivity for α_{2b}-adrenoceptors) attenuated the increase in free water clearance but not the osmolar effect elicited by clonidine (Blandford & Smyth, 1988). These preliminary studies indicated that the actions of clonidine could be separated pharmacologically. Following clonidine, it appeared that the increase in free water clearance was prazosin-sensitive and naltrexone-insensitive, whereas the increase in osmolar clearance was conversely altered (that is, naltrexone-sensitive/prazosin-insensitive).

In the present study, we systematically confirmed these preliminary unpublished observations that the increases in free water clearance and osmolar clearance observed following clonidine could be dissociated pharmacologically with prazosin and naltrexone. This indicated that two sites were involved in the renal response to clonidine. We speculated then that the prazosin-sensitive free water response was mediated by the α_{2b}-subtype and that the other α_2-subtype in the rat kidney, the α_{2a}-subtype, was mediating the increase in osmolar clearance. To rationalize this hypothesis further, the potential role of the α_{2a}-adrenoceptor in mediating the increase in osmolar clearance was investigated with preliminary studies using the relatively selective α_{2a}-adrenoceptor agonist, UK-14,304. These studies determined whether the increase in osmolar clearance following UK-14,304 was naltrexone-sensitive and prazosin-insensitive. Clonidine has also been reported to be a mixed α_2-adrenoceptor/imidazoline receptor agonist (Bousquet *et al.*, 1984). Moxonidine, an imidazoline receptor agonist, was used to determine whether the naltrexone-sensitive increase in osmolar clearance following clonidine or UK-14,304 may have been mediated at imidazoline receptors.

These studies indicated that the ability of clonidine to increase free water and osmolar clearance can be dissociated pharmacologically into two distinct sites, a prazosin-sensitive/naltrexone-insensitive site and a naltrexone-sensitive/prazosin-insensitive site. Moreover, it does not appear to involve the

[1] Author for correspondence.

imidazoline receptor. Based on the prazosin sensitivity of the increase in free water clearance following clonidine and the ability of UK-14,304 and clonidine to induce a naltrexone-sensitive and prazosin-insensitive increase in osmolar clearance, we present the hypothesis that these effects are conceivably mediated by the α_{2b}- and α_{2a}-adrenoceptor subtypes respectively. This theory clearly requires thorough investigation.

Methods

Experimental preparation

The general procedures have been described previously by Blandford & Smyth (1988). Briefly, male Sprague-Dawley rats (200–225 g) were obtained from the University of Manitoba (Charles River Breeding Stock) and cared for according to regional animal care standards protocol. The animals were fed a standard Purina rat chow diet with free access to tap water in cages at 22°C with a 12 h light/dark cycle. Seven to ten days prior to the experiment, the right kidney was removed under ether anaesthesia via a flank incision.

On the day of the experiment, the rats were anaesthetized with pentobarbitone (BDH Chemicals Ltd., Poole, England, 50.0 mg kg^{-1}, i.p.). Additional anaesthetic was administered as required in a bolus dose of 3.0 mg kg^{-1}, i.v. The rats were placed on a Harvard Animal Blanket Control Unit with a rectal thermometer probe and the temperature was set for 37.5°C. A tracheotomy was performed, after which the animals were allowed to breathe spontaneously. The left carotid artery was cannulated with PE-60 tubing and connected to a Statham pressure transducer (Model P23Dc) and a Grass model 5 polygraph for the monitoring of blood pressure. The left jugular vein was cannulated with PE-160 for the infusion of normal saline at 97 $\mu l\ min^{-1}$ and additional anaesthetic as required. A left flank incision was performed and the remaining kidney exposed. The ureter was catheterized to facilitate the collection of urine into pre-weighed vials. Urine volume was determined gravimetrically. Finally, a 31 gauge stainless steel needle was advanced into the renal artery for the infusion of the agonist of interest or vehicle with a Harvard sage pump.

The preparation was allowed to stabilize for 45 min. When necessary, antagonists (naltrexone or prazosin) or vehicle were administered 15 min following the start of the stabilization period as a slow intravenous bolus over 1 min. Immediately following the stabilization period, a 30 min control urine collection was obtained. At this time, the intrarenal infusion (3.4 $\mu l\ min^{-1}$) of agonist (clonidine, moxonidine, or UK-14,304) or vehicle (0.9% saline) was initiated and maintained for the duration of the experiment.

Effects of prazosin or naltrexone on the renal effects of clonidine

Animals were randomly assigned to one of six study groups, each consisting of at least six rats. Group 1, the vehicle control group, received an intrarenal infusion of vehicle at 3.4 $\mu l\ min^{-1}$. Groups 2 and 3 received naltrexone (3.0 mg kg^{-1}) or prazosin (0.15 mg kg^{-1}) alone respectively. Group 4 received an intrarenal infusion of clonidine (1.0 nmol $kg^{-1}\ min^{-1}$). Groups 5 and 6 received pretreatment with prazosin or naltrexone respectively followed by an infusion of clonidine as in group 4.

Table 1 Baseline values obtained before intrarenal clonidine or vehicle infusion

	Con ($n=9$)	*Clon* ($n=9$)	*PZ* ($n=6$)	*Clon+PZ* ($n=6$)	*NX* ($n=6$)	*Clon+NX* ($n=6$)
Blood pressure (mmHg)	119±5	119±6	113±4	106±5	112±3	124±3
Creatinine clearance ($ml\ min^{-1}$)	1.7±0.2	1.9±0.2	1.6±0.1	1.7±0.3	1.8±0.2	1.6±0.1
Urine flow rate ($\mu l\ min^{-1}$)	12±1	22±2**	8±1	7±2	15±3	20±1*
Sodium excretion ($\mu Eq\ min^{-1}$)	1.3±0.2	3.4±0.6**	0.6±0.1	0.4±0.1	2.8±0.9*	4±0.5**
Free water clearance ($\mu l\ min^{-1}$)	−46±4	−45±6	−27±4*	−34±6	−49±7	−66±2*
Osmolar clearance ($\mu l\ min^{-1}$)	59±6	66±7	35±5*	41±7	65±9	85±3**

Con, vehicle control; Clon, clonidine (1.0 nmol $kg^{-1}\ min^{-1}$); PZ, prazosin (0.15 mg kg^{-1}, i.v.); NX, naltrexone (3.0 mg kg^{-1}, i.v.). These values represent the control collection following the stabilization/antagonist pretreatment period.

Table 2 Baseline values before intrarenal moxonidine or vehicle infusion

	Con ($n=6$)	*MOX* ($n=6$)	*NX* ($n=6$)	*MOX+NX* ($n=6$)
Blood pressure (mmHg)	113±7	106±3	107±4	114±5
Creatinine clearance ($ml\ min^{-1}$)	1.1±0.1	1.7±0.2**	1.8±0.2**	1.4±0.1
Urine flow rate ($\mu l\ min^{-1}$)	15.2±2.7	19.5±3.9	16.7±1.2	16.2±2.4
Sodium excretion ($\mu Eq\ min^{-1}$)	2.4±0.7	1.8±0.4	2.2±0.3	1.4±0.3
Free water clearance ($\mu l\ min^{-1}$)	−50±6	−45±6	−63±2	−38±5
Osmolar clearance ($\mu l\ min^{-1}$)	65±8	65±8	80±3	54±7

Con, vehicle control; MOX, moxonidine (3.0 nmol $kg^{-1}\ min^{-1}$); NX, naltrexone (3.0 mg kg^{-1}, i.v.). These values represent the control collection following the stabilization/antagonist pretreatment period.

Effects of naltrexone on the osmolar response to moxonidine

Animals were randomly assigned to one of four study groups, each consisting of at least six rats. Group 1, the control group, received an intrarenal infusion of vehicle at 3.4 μl min^{-1}. Group 2 received naltrexone (3.0 mg kg^{-1}) alone. Group 3 received an intrarenal infusion of moxonidine (3.0 nmol kg^{-1} min^{-1}). Group 4 received pretreatment with naltrexone, followed by moxonidine as in group 3.

Effect of prazosin or naltrexone on the renal actions of UK-14,304

The possibility that the α_{2a}-adrenoceptor subtype mediates solute handling in the kidney was investigated by use of UK-14,304, a purported selective α_{2a}-subtype agonist. Animals were randomly assigned to one of six study groups, each consisting of at least six rats. Group 1 received an intrarenal infusion of normal saline at 3.4 μl min^{-1}. Groups 2 and 3 received naltrexone (3.0 mg kg^{-1}) or prazosin (0.15 mg kg^{-1}) alone respectively. Group 4 received an intrarenal infusion of UK-14,304 (1.0 nmol kg^{-1} min^{-1}). Groups 5 and 6 received pretreatment with prazosin or naltrexone respectively, followed by an infusion of UK-14,304 as in group 4.

Sample analysis

At the end of the experiment, a blood sample was collected through the carotid artery catheter. Dye was injected through the renal artery line to confirm proper position of the needle. Creatinine levels in the urine and plasma were measured with a Beckman Creatinine 2 Analyser. Urine and plasma osmolalities were determined with a Precision Systems Micro Osmometer. The sodium concentrations in urine and plasma were measured with a Nova Electrolyte Analyser (13+).

Statistical analysis

Data are presented as the mean ± standard error (s.e.). Data were analysed by repeated measures of analysis of variance (ANOVA) using the SAS software, version 6.07. Significant interactions were further analysed by a Fisher's least squares difference multiple comparison test. Significance is denoted in the figures with * representing $P<0.05$ and ** representing $P<0.01$. For purposes of presentation, absolute values of blood pressure and creatinine clearance have been presented for all three collection periods so that comparison to control within each collection period is possible. For urine flow rate, sodium excretion and free water and osmolar clearance, the data have been expressed graphically as the absolute change from the first to final collection period. This allowed the determination of the magnitude of the change for each variable within the different groups.

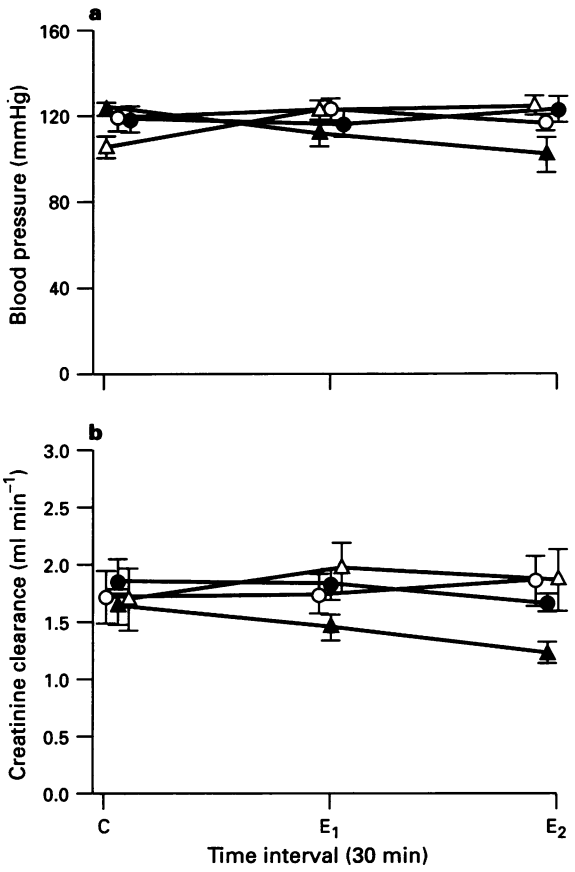

Figure 1 Effects of clonidine in the presence and absence of naltrexone or prazosin on (a) blood pressure and (b) creatinine clearance in the rat. The different pharmacological interventions are illustrated as follows: (○) control; (●) clonidine; (△) clonidine and prazosin; (▲) clonidine and naltrexone. Each group represents the mean ± s.e. of at least 6 experiments. C denotes the absolute values measured during the control collection prior to the infusion of clonidine or saline control. E_1 and E_2 denote the absolute values of the two post clonidine or saline infusion collections.

Table 3 Baseline values before intrarenal UK-14,304 or vehicle infusion

	Con ($n=9$)	*UK* ($n=8$)	*PZ* ($n=9$)	*UK+PZ* ($n=8$)	*NX* ($n=6$)	*UK+NX* ($n=6$)
Blood pressure (mmHg)	118±5	123±3	98±5**	114±4	118±4	116±2
Creatinine clearance (ml min^{-1})	1.5±0.2	1.3±0.2	1.8±0.1	1.5±0.1	1.7±0.1	1.6±0.1
Urine flow rate (μl min^{-1})	15±2	20±3	9±1*	9±2	17±4	11±1
Sodium excretion (μEq min^{-1})	1.6±0.3	2.2±0.3	0.7±0.2*	0.9±0.2	1.8±0.6	0.7±0.1
Free water clearance (μl min^{-1})	−34±3	−42±3	−34±4	−40±5	−41±4	−41±3
Osmolar clearance (μl min^{-1})	49±5	61±4	43±5	50±6	57±5	52±3

Con, vehicle control; UK (1.0 nmol kg^{-1} min^{-1}); PZ, prazosin (0.15 mg kg^{-1}, i.v.); NX, naltrexone (3.0 mg kg^{-1}, i.v.). These values represent the control collection following the stabilization/antagonist pretreatment period.

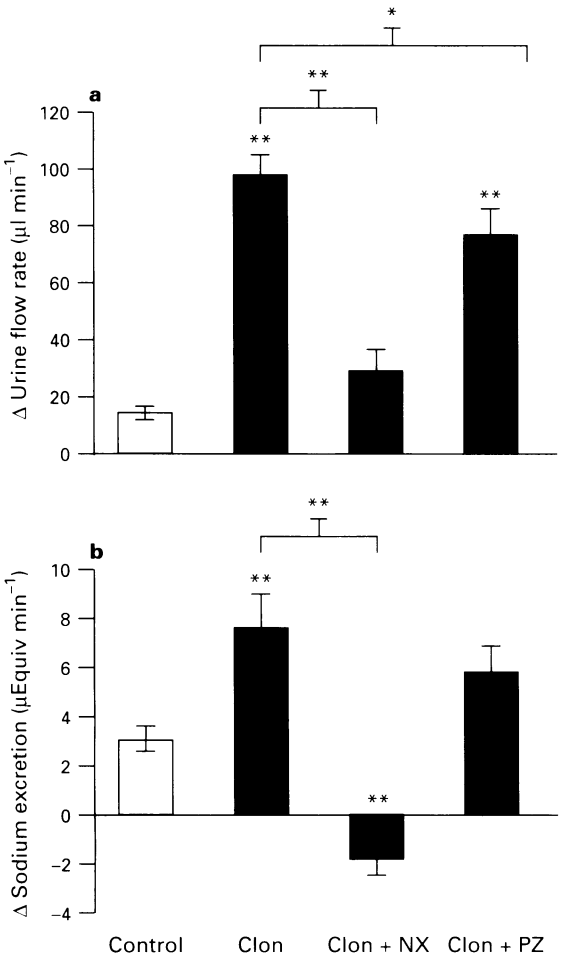

Figure 2 Effects of clonidine in the presence and absence of naltrexone or prazosin on (a) urine flow rate and (b) sodium excretion in the rat. Clon, clonidine; Clon + NX, clonidine following naltrexone pretreatment; Clon + PZ, clonidine following prazosin pretreatment. Each group represents the mean ± s.e. of the delta (final collection minus baseline) values of at least 6 experiments. $^{**}P<0.01$ versus control; $^{*}P<0.05$ and $^{**}P<0.01$ respectively between groups.

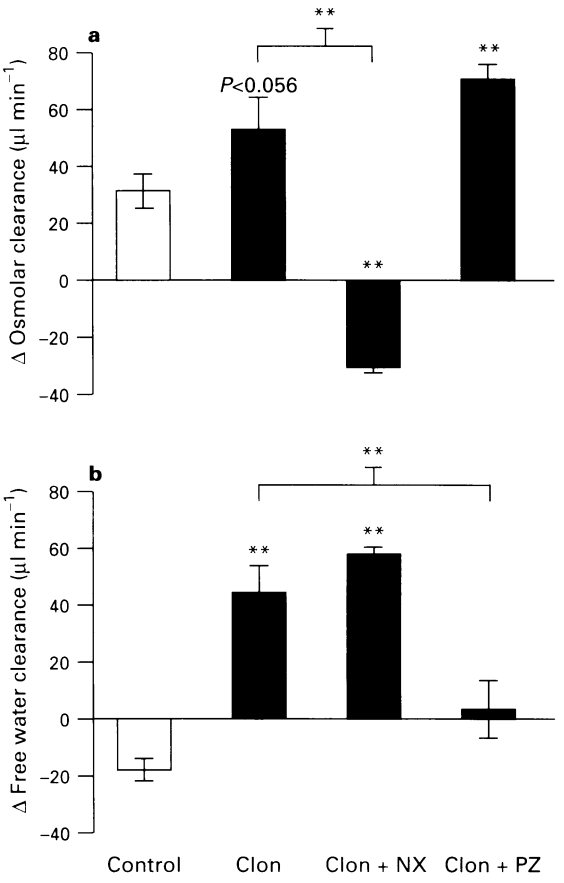

Figure 3 Effects of clonidine in the presence and absence of naltrexone or prazosin on (a) osmolar and (b) free water clearance in the rat. Clon, clonidine; Clon + NX, clonidine following naltrexone pretreatment; Clon + PZ, clonidine following prazosin pretreatment. Each group represents the mean ± s.e. of the delta (final collection minus baseline) values of at least 6 experiments. $^{**}P<0.01$ versus control; $^{**}P<0.01$ between groups.

Drugs

Clonidine (Sigma Chemical Co., St. Louis, MO, U.S.A.), prazosin (Sigma Chemical Co., St. Louis, MO, U.S.A.), naltrexone (Sigma Chemical Co., St. Louis, MO, U.S.A.), moxonidine (Beiersdorf, AG, Hamburg, Germany) and UK-14,304 (5-bromo-6-[2-imidazoline-2-ylamino]-quinoxaline; Pfizer Central Research) were used in the present studies.

Results

Preparation controls

Data from the first collection period were analysed to determine the conformity between groups following the surgery. Baseline values of blood pressure, creatinine clearance as well as all parameters of interest are shown in Tables 1, 2 and 3. Due to minor differences between baseline groups, the renal data (urine flow rate, sodium excretion, osmolar and free water clearances) have been presented as the difference between baseline and final collection values (that is, deltas) to reveal the different magnitudes of responses between groups. Blood pressure and creatinine clearance have been presented as absolute values to illustrate similarity between groups during each collection period.

Effects of prazosin or naltrexone on the renal response to clonidine

Blood pressure and creatinine clearance were unaltered (Figure 1) by the intrarenal infusion of clonidine in the presence or absence of naltrexone or prazosin. Similarly, naltrexone or prazosin pretreatment alone failed to alter these parameters (data not shown).

Clonidine increased urine flow rate and sodium excretion (Figure 2) which was reflected by increases in osmolar and free water clearance (Figure 3). Pretreatment with prazosin (relatively selective α_{2b}-adrenoceptor subtype antagonist) selectively decreased the clonidine-induced increase in free water clearance but failed to alter the increase in osmolar clearance (Figure 3). Pretreatment with naltrexone (non-selective opioid receptor antagonist) attenuated the increase in urine flow rate and sodium excretion following clonidine (Figure 2). This attenuation was secondary to a selective decrease in the clonidine-induced increase in osmolar clearance. Naltrexone failed to alter the increase in free water clearance following clonidine (Figure 3).

Effect of naltrexone on the osmolar response to moxonidine

Blood pressure was slightly increased by moxonidine during the third collection period as compared to the control group (Figure 4). Blood pressure was not altered in the groups receiving naltrexone alone or naltrexone with moxonidine. Although baseline creatinine clearance differed between groups, within each group the creatinine clearance did not change over

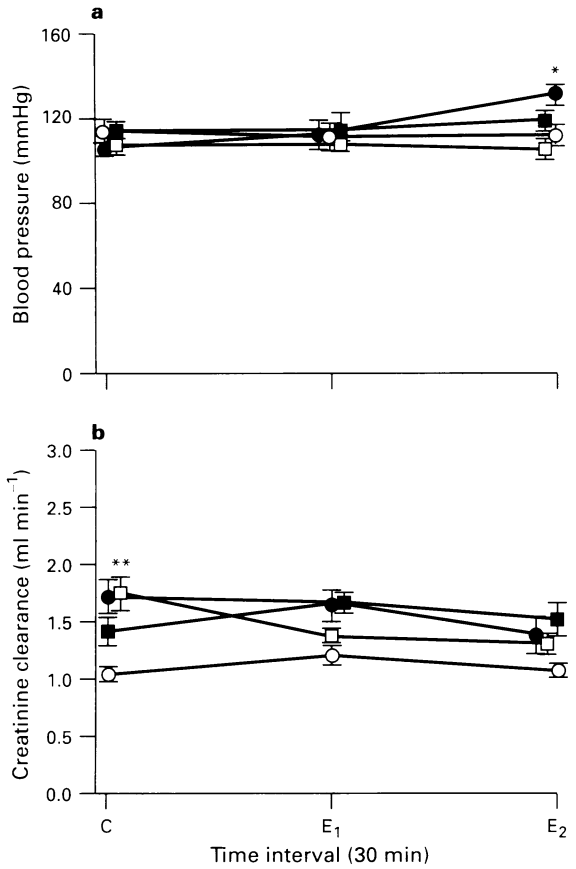

Figure 4 Effects of naltrexone, moxonidine, or moxonidine with naltrexone pretreatment on (a) blood pressure and (b) creatinine clearance in the rat. The different pharmacological interventions are illustrated as follows: (○) control; (●) naltrexone; (□) moxonidine; (■) moxonidine and naltrexone. Each group represents the mean ± s.e. of at least 6 experiments. *$P<0.05$ versus control. C denotes the absolute values measured during the control collection prior to the infusion of moxonidine or saline control. E_1 and E_2 denote the absolute values of the two post moxonidine or saline infusion collections.

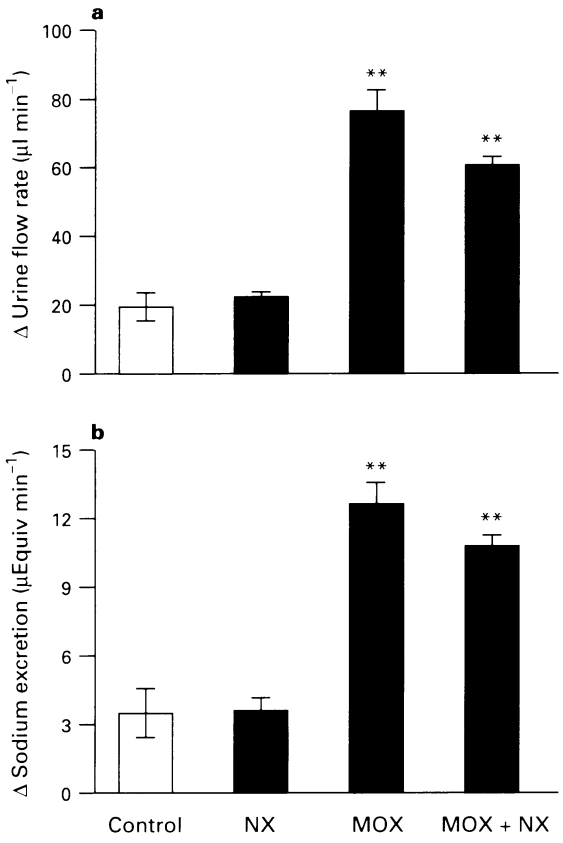

Figure 5 Effects of moxonidine in the presence and absence of naltrexone on (a) urine flow rate and (b) sodium excretion in the rat. NX, naltrexone; MOX, moxonidine; MOX + NX, moxonidine following naltrexone pretreatment. Each group represents the mean ± s.e. of the delta (final collection minus baseline) values of at least 6 experiments. **$P<0.01$ versus control.

the duration of the experiment (Figure 4). Moxonidine increased urine flow rate and sodium excretion (Figure 5). This increase was reflected by an increase in osmolar clearance with a decrease in free water clearance (Figure 6). Pretreatment with naltrexone failed to alter the ability of moxonidine to increase urine flow rate, sodium excretion, and osmolar clearance.

Effects of prazosin or naltrexone on the renal response to UK-14,304

Blood pressure and creatinine clearance were unaltered by experimental intervention (Figure 7). Intrarenal infusion of UK-14,304 elicited an increase in urine flow rate and sodium excretion (Figure 8). This response was due solely to an increase in osmolar clearance since free water clearance remained unaltered (Figure 9). Prazosin pretreatment failed to alter the renal response to UK-14,304 significantly (Figures 8 and 9). On the other hand, naltrexone pretreatment abolished the increases in urine flow rate and sodium excretion observed following UK-14,304 (Figure 8) as reflected by an attenuation of the increase in osmolar clearance (Figure 9). Naltrexone pretreatment alone did not alter baseline renal function (Table 3).

Discussion

In anaesthetized rats, the intrarenal infusion of clonidine has been reported to increase urine flow rate. This response reflects an increase in free water clearance and osmolar clearance (Blandford & Smyth, 1988; 1990; 1991). Studies from our laboratory have suggested that these effects were mediated by at least two distinct sites. In the rat, low doses of clonidine produced an increase only in free water clearance while higher doses increased both free water and osmolar clearance (Blandford & Smyth, 1988). Studies with indomethacin pretreatment were also consistent with the two-site hypothesis. Indomethacin potentiated the increase in osmolar clearance but had no effect or attenuated the increase in free water clearance (Blandford & Smyth, 1991). If clonidine was acting at only one site, the anticipated effect of indomethacin pretreatment would be a similar action on both osmolar and free water clearance (that is, indomethacin would either decrease or increase both parameters).

The present investigation provides two further pharmacologically distinct lines of evidence (dissociation by prazosin and by naltrexone) which indicate that two different sites are involved in the renal effects of clonidine. Prazosin significantly decreased the ability of clonidine to increase free water clearance without altering the influence of clonidine on osmolar clearance. In contrast, naltrexone abolished the clonidine-induced increase in osmolar clearance but failed to alter the effect of clonidine on free water clearance.

In the present study, the selective blockade of the increase in free water clearance by prazosin suggested that this effect was mediated by α_1-adrenoceptors. However, the intravenous administration of the α_1-selective agonist, cirazoline, has been demonstrated to have no effect on urine flow rate, free water or osmolar clearance in the rat (Gellai & Ruffulo, 1987). We subsequently speculated that the α_{2b}-adrenoceptor subtype was mediating the prazosin-sensitive free water response to cloni-

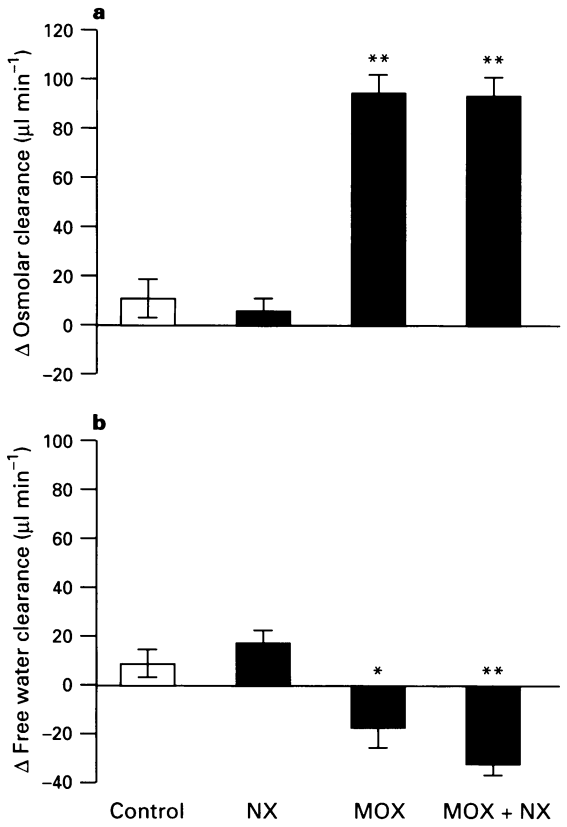

Figure 6 Effects of moxonidine in the presence and absence of naltrexone on (a) osmolar and (b) free water clearances in the rat. NX, naltrexone; MOX, moxonidine; MOX + NX, moxonidine following naltrexone pretreatment. Each group represents the mean ± s.e. of the delta (final collection minus baseline) values of at least 6 experiments. $*P<0.05$ and $**P<0.01$ versus control.

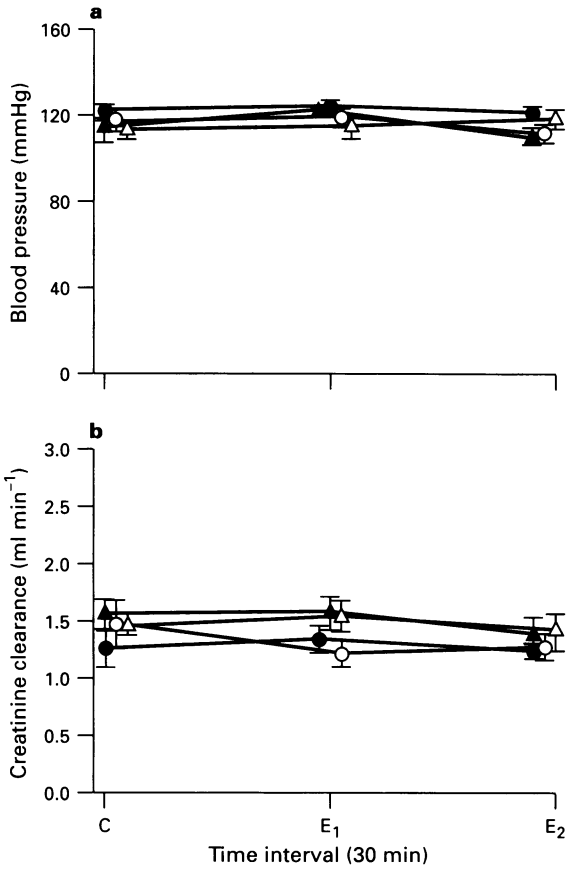

Figure 7 Effects of UK-14,304 in the absence of naltrexone or prazosin on (a) blood pressure and (b) creatinine clearance in the rat. The different pharmacological interventions are illustrated as follows: (○) control; (●) UK-14,304; (△) UK-14,304 and prazosin; (▲) UK-14,304 and naltrexone. Each group represents the mean ± s.e. of at least 6 experiments. C denotes the absolute values measured during the control collection prior to the infusion of UK-14,304 or saline control. E_1 and E_2 denote the absolute values of the two post UK-14,304 or saline infusion collections.

dine. Although prazosin is clearly an α_1-adrenoceptor antagonist, it has also been shown to display a greater selectivity for the α_{2b}-adrenoceptor subtype over other α_2-subtypes (Uhlén & Wikberg, 1991a). Based on the low affinity of prazosin for the α_{2a}-subtype and high affinity for the α_{2b}-subtype, the initial distinction between these two subtypes was discerned (Bylund 1985; Nahorski *et al.*, 1985). Thus, we hypothesize that the free water response to clonidine was mediated by the α_{2b}-adrenoceptor subtype. However, the naltrexone-sensitive increase in osmolar clearance produced by clonidine was unaffected by prazosin pretreatment and therefore appeared independent of both α_1-adrenoceptor and α_{2b}-adrenoceptor subtype stimulation.

The site responsible for the naltrexone-sensitive increase in osmolar clearance following clonidine is not clear. The distribution of α_2-adrenoceptor mRNA in the rat kidney as detected by *in situ* hybridization has been reported. $\alpha_{2a/d}$- and α_{2b}-Adrenoceptor mRNAs were widely distributed whereas α_{2c}-subtype mRNA was distributed to a much lesser extent (Meister *et al.*, 1994). The α_{2d}-adrenoceptor has been proposed as the rat analogue of the human α_{2a}-subtype (Uhlén & Wikberg, 1991b; Uhlén *et al.*, 1993). It does not appear as if the α_{2c}-transcripts are translated as Uhlén & Wikberg (1991a,b) have identified only the α_{2a}- and α_{2b}-adrenoceptor subtypes in the kidney via radioligand binding studies. By deduction then, if only the α_{2a}- and α_{2b}-subtypes are found in the rat kidney, clonidine may have increased osmolar clearance by stimulating the α_{2a}-adrenoceptor subtype.

UK-14,304 has been found to be a relatively selective α_{2a}-adrenoceptor agonist. For example, UK-14,304 was shown to have up to a 100 fold greater potency for inhibiting adenylate cyclase at the α_{2a}-subtype versus the α_{2b}-subtype in two cell lines. These included the HT29 cell line which selectively expresses the α_{2a}-subtype and the NG108 cell line which expresses the α_{2b}-subtype (Bylund & Ray-Prenger, 1989). We therefore determined the ability of a selective α_{2a}-adrenoceptor agonist, UK-14,304 (MacKinnon *et al.*, 1994) to increase osmolar clearance selectively and, in turn, if this increase was naltrexone-sensitive and prazosin-insensitive. In the present study, a low dose of intrarenal infusion of UK-14,304 elicited an increase in urine flow rate which was due to an increase in osmolar clearance. No effect on free water clearance was observed. This increase in osmolar clearance was abolished by pretreatment with naltrexone and unaltered by pretreatment with prazosin. These findings do not prove but are consistent with the naltrexone-sensitive increase in osmolar clearance following clonidine or UK-14,304 involving the α_{2a}-adrenoceptor subtype.

Clonidine and UK-14,304 have been identified as mixed agonists, stimulating α_2-adrenoceptors as well as imidazoline receptors (Bousquet *et al.*, 1984; Ernsberger *et al.*, 1988; Tibirica *et al.*, 1991; Hieble & Ruffolo, 1992). Renal imidazoline receptor stimulation has been shown to increase urine flow rate by increasing osmolar but not free water clearance (Allan *et al.*, 1993). Hence, it was unclear whether the intrarenal infusion of clonidine or UK-14,304 was increasing osmolar clearance by stimulating α_2-adrenoceptors and/or imidazoline receptors. In the present study, moxonidine was selected due to a reported 600 fold higher affinity for imidazoline receptors than for α_2-adrenoceptors in the rat kidney (Ernsberger *et al.*, 1993). The intrarenal infusion of mox-

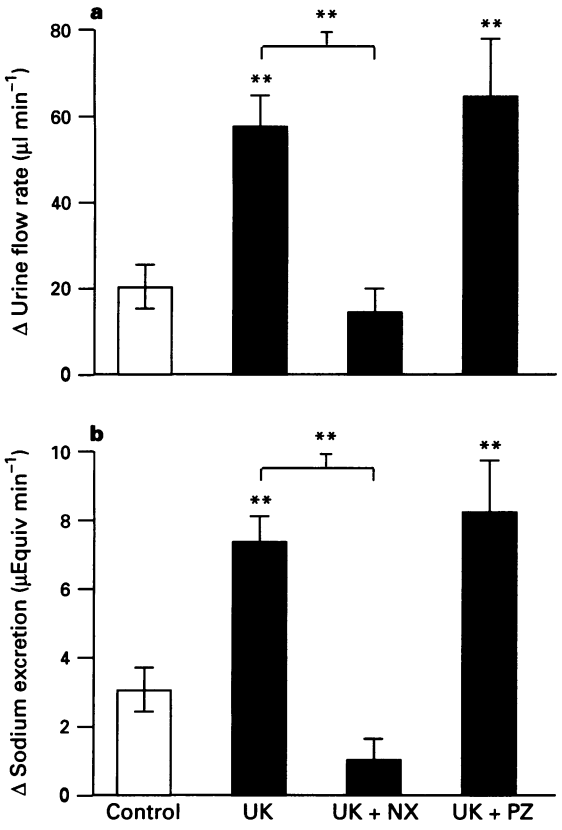

Figure 8 Effects of UK-14,304 in the presence and absence of naltrexone or prazosin on (a) urine flow rate and (b) sodium excretion in the rat. UK, UK-14,304; UK+NX, UK-14,304 following naltrexone pretreatment; UK+PZ, UK-14,304 following prazosin pretreatment. Each group represents the mean±s.e of the delta (final collection minus baseline) values of at least 6 experiments. $^{**}P<0.01$ versus control $^{**}P<0.01$ between groups.

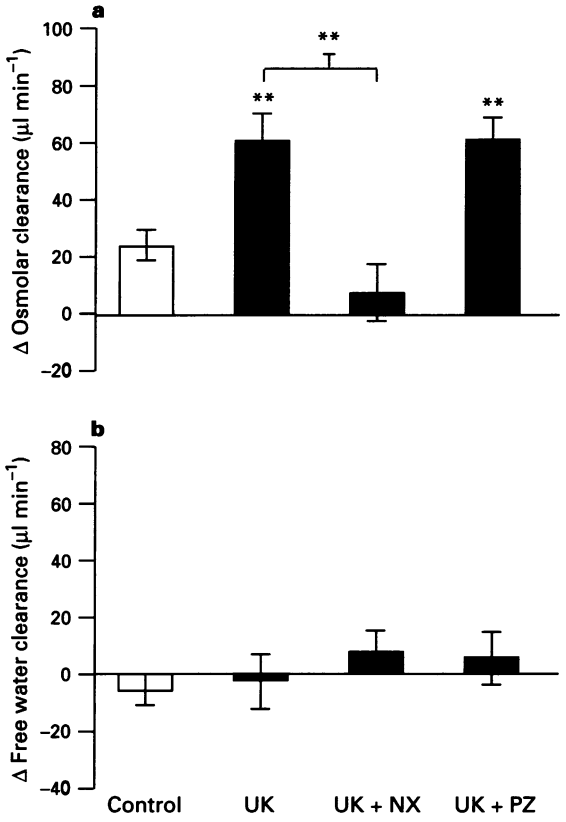

Figure 9 Effects of UK-14,304 in the presence and absence of naltrexone or prazosin on osmolar and free water clearance in the rat. UK, UK-14,304; UK+NX, UK-14,304 following naltrexone pretreatment; UK+PZ, UK-14,304 following prazosin pretreatment. Each group represents the mean±s.e. of the delta (final collection minus baseline) values of at least 6 experiments. $^{**}P<0.01$ versus control; $^{**}P<0.01$ between groups.

onidine, as with UK-14,304, produced an increase in urine flow rate, as reflected by an increase in osmolar clearance without any increase in free water clearance. In contrast to the findings with clonidine and UK-14,304, the osmolar response to moxonidine was unaffected by naltrexone. This indicated that the naltrexone-sensitive increase in osmolar clearance did not involve imidazoline receptors. Furthermore, clonidine and UK-14,304 probably increased osmolar clearance by a mechanism distinct from that stimulated by imidazoline receptor activation. In support of this, previous studies in our laboratory demonstrated that rauwolscine (selective α_2-adrenoceptor antagonist) blocked the renal effects of clonidine but not those of moxonidine. In contrast, idazoxan (selective imidazoline receptor antagonist) blocked the renal effects of moxonidine but not those of clonidine (Allan *et al.*, 1993). Most conceivably, therefore, α_2-adrenoceptors mediated the increase in osmolar clearance produced by clonidine and UK-14,304.

The pharmacological data presented here allow the conclusion that two sites are involved; the nature of these two sites requires further study. In fact, the involved mechanisms may be extra-tubular, related to renal nerve activity, or due to resetting mechanisms of unidentified origin. However, based on (a) the insensitivity of the clonidine-induced osmolar response to prazosin, (b) the ability of UK-14,304 to increase selectively osmolar clearance, (c) the insensitivity of the moxonidine-induced osmolar response to naltrexone, and finally (d) the report that only the α_{2a}- and α_{2b}-subtypes exist in the rat kidney, we suggest the following hypothesis: the renal α_{2a}-subtype mediates osmolar clearance whereas the α_{2b}-subtype mediates free water clearance.

In conclusion, clonidine increased urine flow rate by increasing both free water clearance and osmolar clearance. This response to clonidine was dissociated pharmacologically wherein the free water effect was prazosin-sensitive/naltrexone-insensitive and the osmolar effect was naltrexone-sensitive/prazosin-insensitive. These findings would be consistent with clonidine-induced osmolar clearance and free water clearance being mediated at two distinct sites and/or receptors. We also submit the hypothesis that the effects of clonidine on free water clearance and osmolar clearance involve specific activation of the α_{2a}- and α_{2b}-adrenoceptors respectively. However, it must be emphasized that this conjecture requires further study. The prazosin-sensitivity of the free water clearance response potentially indicated a role for the α_{2b}-adrenoceptor. The site mediating the osmolar clearance response also remains unclear although inability of naltrexone to block the actions of moxonidine indicates that the imidazoline receptor may be excluded as a possibility. The α_{2a}-adrenoceptor may be the subtype involved in this osmolar response since UK-14,304, with purported selectivity for the α_{2a}-subtype, produced a naltrexone-sensitive/prazosin-insensitive increase in osmolar clearance.

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