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# Antagonism by idazoxan at low dose but not high dose, of the natriuretic action of moxonidine

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1 Recent studies concerning the imidazoline receptor have utilized idazoxan as a specific imidazoline receptor antagonist. The aim of the present study was to describe the *in vivo* effects of various doses of idazoxan on renal function, in the presence and absence of moxonidine, an $I_1$ imidazoline receptor agonist.

2 In anaesthetized, unilaterally nephrectomized (7 to 10 days) Sprague Dawley rats, an intrarenal infusion of moxonidine (3 nmol $kg^{-1}$ $min^{-1}$) increased urine flow rate, sodium excretion and osmolar clearance without altering free water clearance. Pretreatment with intravenous idazoxan at 0.1 and 0.3 mg $kg^{-1}$ produced a dose-related decrease in the renal actions of moxonidine. However, a higher dose of idazoxan (1 mg $kg^{-1}$) was not as effective as the 0.3 mg $kg^{-1}$ dose in blocking the effects of moxonidine.

3 In a separate series of experiments, the direct renal actions of idazoxan alone were investigated. Idazoxan at 0.3 mg $kg^{-1}$ failed to alter urine flow rate and sodium excretion. However, idazoxan at 1 mg $kg^{-1}$ produced a significant increase in urine flow rate and sodium excretion in association with an increase in osmolar clearance.

4 These results do not prove but are consistent with low doses of idazoxan antagonizing the sites stimulated by moxonidine (renal imidazoline receptors). However, at higher doses, idazoxan may function as a partial agonist and/or interact with other receptors to increase urine flow rate, independent of imidazoline receptor blockade. These studies underscore the importance of the dose of idazoxan administered when this antagonist is used as a tool to investigate imidazoline receptors.

**Keywords:** Moxonidine; clonidine; $\alpha_2$-adrenoceptor; imidazoline receptor

## Introduction

A number of studies have used rauwolscine and idazoxan as specific $\alpha_2$-adrenoceptor antagonists. However, radioligand binding studies demonstrated that these two antagonists in fact labelled two distinct populations of receptors (Boyajian *et al.*, 1987; Coupry *et al.*, 1987). Subsequently, it was found that [$^3$H]-idazoxan labelled a non-adrenoceptor site which had a higher affinity for imidazoline-based compounds than for catecholamines (phenylethylamine based compounds) (Bricca *et al.*, 1989; Michel *et al.*, 1989; 1990; Ernsberger *et al.*, 1990). These non-adrenoceptor sites were later termed imidazoline sites (Ernsberger *et al.*, 1992; Reis *et al.*, 1992). Idazoxan has been demonstrated to possess at least a 50 fold greater selectivity for these imidazoline sites over $\alpha_2$-adrenoceptors (Lehmann *et al.*, 1989).

In recent years, a number of studies have utilized idazoxan as a specific antagonist to describe the function of imidazoline receptor stimulation *in vivo*. Idazoxan has been shown to antagonize the blood pressure lowering effect of clonidine in the nucleus reticularis lateralis (Tibirica *et al.*, 1991), the diuretic action of moxonidine (Allan *et al.*, 1993) and the blood pressure lowering actions of rilmenidine (Gomez *et al.*, 1991). However, in preliminary studies in our laboratory, we observed that increasing the dose of idazoxan failed to increase the degree of blockade. In fact, increasing the dose decreased the level of blockade which had been observed at lower doses. These initial observations were consistent with the doses of idazoxan acting as either a partial agonist or at another independent receptor site. Similar conclusions have been presented by others with very high concentrations in a pithed rat preparation (Paciorek & Shepperson, 1983) and in isolated vascular strips (Schwietert *et al.*, 1992).

Idazoxan has become an important pharmacological tool for the investigation of the imidazoline receptor. Consequently, it was considered that the dose-response relationship between the imidazoline receptor antagonist, idazoxan and an imidazoline receptor agonist should be investigated systematically in a whole animal preparation. In the present study we describe the ability of increasing doses of idazoxan to antagonize the renal actions of moxonidine, an imidazoline agonist which has been demonstrated to possess a 600 fold greater selectivity for these sites over $\alpha_2$-adrenoceptors (Ernsberg *et al.*, 1993). The present findings suggest that at moderate doses *in vivo*, idazoxan may no longer function as an imidazoline receptor antagonist. These findings underscore the importance of dose selection when idazoxan is used as a tool to identify the physiological function of imidazoline recetors and may help to explain some of the confusion and conflicting conclusions in this area of investigation.

## Methods

The general methodology has been described previously by Blandford & Smyth (1988a,b; 1990). 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. They 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.

The right kidney was removed via a flank incision under ether anaesthesia 7-10 days prior to the experiment. On the study day, rats were anaesthetized with 50 mg $kg^{-1}$, (i.p.)

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pentobarbitone (BDH Chemicals, Poole, England). Further anaesthesia during the course of the experiment was maintained with intravenous pentobarbitone (5 mg $kg^{-1}$) as required. The animal was placed on a Harvard Animal Blanket Control Unit with a rectal thermometer probe set for 37.5°C. A tracheotomy was performed. The left carotid artery was cannulated with PE-60 tubing and connected to a Statham pressure transducer (Model P23Dc) and Grass Model 5 Polygraph for the monitoring of blood pressure and heart rate. The left jugular vein was cannulated with PE-160 for the infusion of normal saline at 97 $\mu l\ min^{-1}$ and administration of intravenous idazoxan. A left flank incision was performed and the remaining kidney exposed. The ureter was catheterized for the timed collection of urine into pre-weighed vials. Urine volume was determined gravimetrically. A 31 gauge stainless steel needle was inserted into the renal artery and secured with glue for later infusion of saline vehicle or moxonidine. Following surgery, the preparation was allowed to stabilize for 45 min after which 5 consecutive 15 min urine collections were made in pre-weighed tubes.

Two series of experiments were conducted. In the first series of experiments, the effects of idazoxan on the renal actions of moxonidine were determined. Saline vehicle or idazoxan (0.1, 0.3 and 1 mg $kg^{-1}$) was administered (0.2 ml over 2 min) 30 min into the stabilization period. Immediately following the first urine collection, an intrarenal infusion (3.4 $\mu l\ min^{-1}$) of saline vehicle or moxonidine (3 nmol $kg^{-1}\ min^{-1}$) was begun and maintained for the duration of the experiment. The 5 groups in this series were: saline control, moxonidine alone, moxonidine plus 0.1 mg $kg^{-1}$ idazoxan, moxonidine plus 0.3 mg $kg^{-1}$ idazoxan and moxonidine plus 1 mg $kg^{-1}$ idazoxan. In the second series of experiments, the effects of idazoxan alone were determined. Saline vehicle or idazoxan (0.3 or 1 mg $kg^{-1}$) was administered (0.2 ml over 2 min) 30 min into the stabilization period. Immediately after the first urine collection, saline was infused into the renal artery at 3.4 $\mu l\ min^{-1}$ and this infusion was maintained for the duration of the experiment. The three groups in this series were: saline control, 0.3 mg $kg^{-1}$ idazoxan and 1 mg $kg^{-1}$ idazoxan.

At the end of the experiment, a sample of blood was collected through the carotid line and the plasma frozen. Methylene blue was injected through the renal arterial line to confirm proper positioning of the needle. Urine and plasma creatinine were measured with a Precision Systems Micro Osmometer. Urine and plasma concentrations of sodium were determined by a Beckman Klina Flame Photometer.

### *Statistical analysis*

Each experimental group consisted of 6–8 rats. Data are presented as the mean ± the standard error of the mean (s.e. mean). Data were analyzed by repeated measures analysis of variance. Significant interactions were further analyzed by a one way ANOVA Fisher's least squares difference multiple comparison test (Winer, 1971). *denotes a $P$ value of $<0.05$ as compared to the saline control group and -*- denotes a $P$ value of $<0.05$ between designated groups. The third, fourth and fifth collection periods were found to demonstrate the same significant differences. For the purpose of presentation, data from the fifth collection period are shown on the graphs since this period is representative of differences observed between groups.

## Results

The first urine collection for each experiment served as a preparation control (data not shown). This allowed evaluation of baseline renal function for each experiment and the determination of altered values secondary to the surgical procedure. In all groups studied, the measured parameters were not different between the experimental groups prior to the administration of the moxonidine. For clarity of presentation, only data from the fifth urine collection are presented in detail as this is representative of differences reported between groups.

### *Effect of idazoxan on the renal actions of moxonidine*

Moxonidine (3 nmol $kg^{-1}\ min^{-1}$) alone, or in the presence of increasing concentrations of idazoxan, failed to alter significantly blood pressure or creatinine clearance as compared to the group receiving the saline vehicle (Figure 1). The infusion of moxonidine produced a marked increase in urine volume, sodium excretion (Figure 2) and osmolar clearance without changing free water clearance (Figure 3). Intravenous idazoxan at 0.1 and 0.3 mg $kg^{-1}$ produced a dose-related inhibition of the effects of moxonidine on urine volume, sodium excretion (Figure 2) and osmolar clearance (Figure 3). In the presence of 0.3 mg $kg^{-1}$ idazoxan, moxonidine failed to have any effect on urine volume and sodium excretion. However, at the next highest dose of idazoxan (1 mg $kg^{-1}$), moxonidine appeared to be effective since a significant increase in urine volume and sodium excretion (Figure 2) and osmolar clearance (Figure 3) was observed.

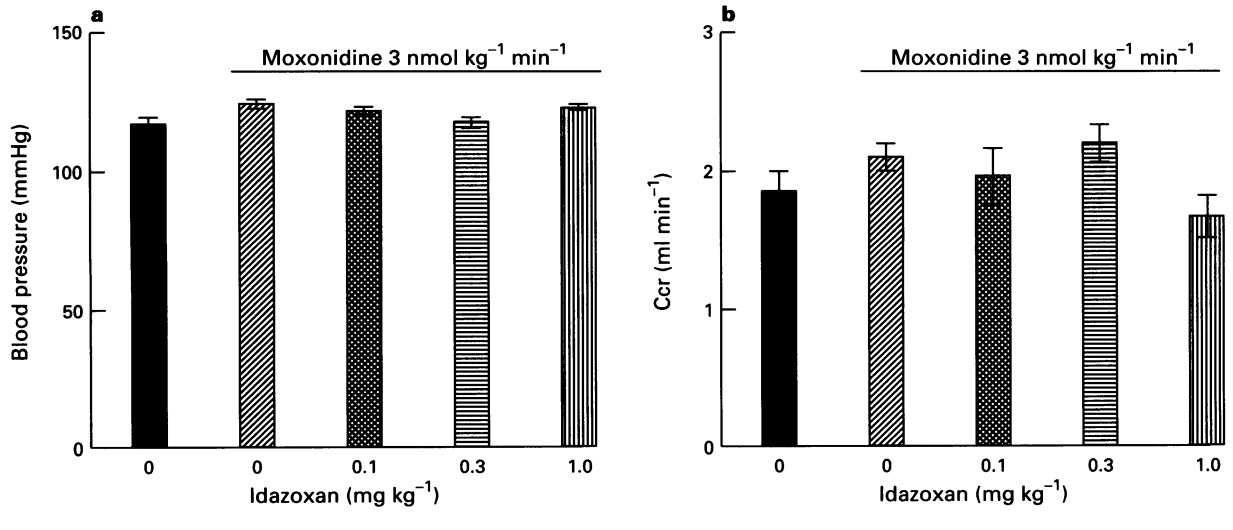


**Figure 1** The effects of intrarenal moxonidine (3 nmol $kg^{-1}\ min^{-1}$) infusion (3.4 $\mu l\ min^{-1}$) on mean arterial blood pressure (a) and creatinine clearance (Ccr) (b) in the absence and presence of increasing doses of intravenous idazoxan. All the data presented as the mean ± s.e.mean.

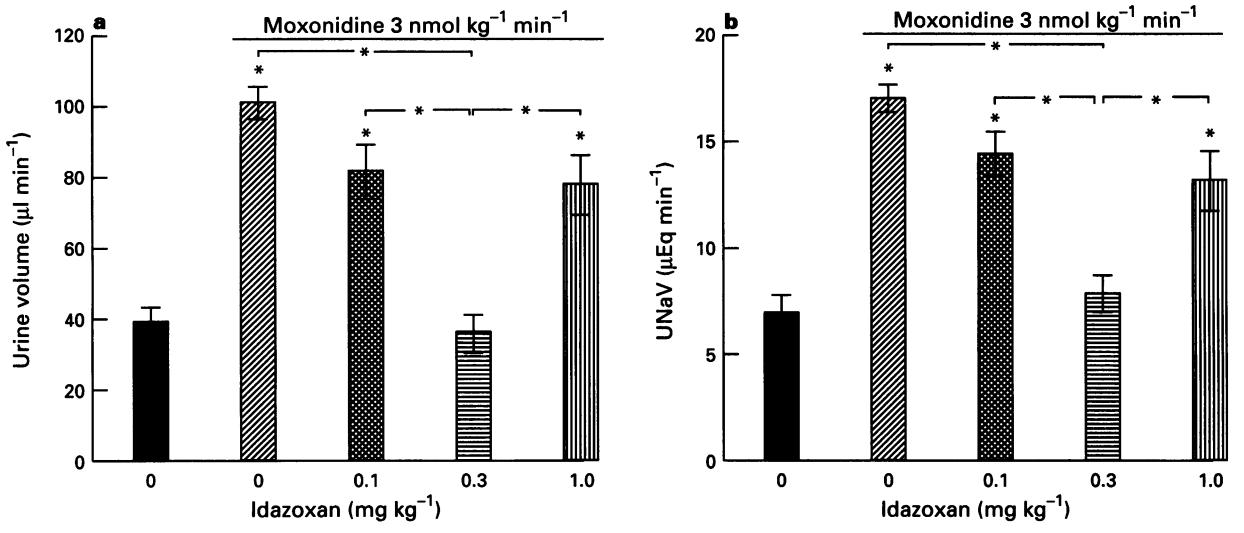


**Figure 2** The effects of intrarenal moxonidine (3 nmol kg$^{-1}$ min$^{-1}$) infusion (3.4 µl min$^{-1}$) on urine volume (a) and sodium excretion (UNaV) (b) in the absence and presence of increasing doses of intravenous idazoxan. All the data presented as the mean ± s.e.mean *$P$<0.5 as compared with the saline vehicle control group (solid column) and -*- denotes a $P$ value of <0.05 between designated groups.

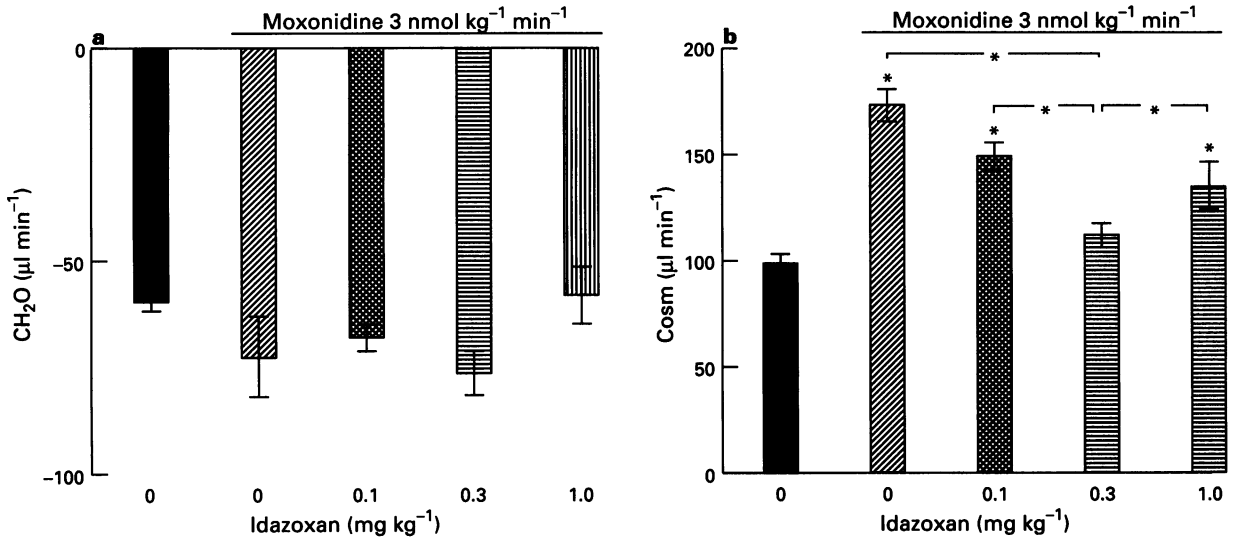


**Figure 3** The effects of intrarenal moxonidine (3 nmol kg$^{-1}$ min$^{-1}$) infusion (3.4 µl min$^{-1}$) on free water ($CH_2O$) clearance and osmolar clearance (Cosm) (b) in the absence and presence of increasing doses of intravenous idazoxan. All the data presented as the mean ± s.e.mean *$P$<0.05 as compared with the saline vehicle control group (solid column) and -*- denotes a $P$ value of <0.05 between designated groups.

### *Renal actions of idazoxan*

Intravenous infusion of idazoxan over 2 min failed to alter blood pressure or creatinine clearance significantly, although a modest increase in blood pressure was observed at a dose of 1 mg kg$^{-1}$ (Figure 4). Idazoxan at 0.3 mg kg$^{-1}$, which antagonized the renal actions of moxonidine (Figures 2 and 3) failed to alter significantly urine flow rate, sodium excretion (Figure 5), osmolar clearance or free water clearance (Figure 6). The highest dose of idazoxan investigated (1 mg kg$^{-1}$), which apparently failed to alter the response to moxonidine (Figures 2 and 3), alone produced a significant increase in urine volume, sodium excretion (Figure 5) and osmolar clearance (Figure 6).

## Discussion

Idazoxan was introduced as a competitive $\alpha_2$-adrenoceptor antagonist (Freedman & Aghajanian, 1984; Dabire, 1986). Recently idazoxan has been found to label specifically non-adrenoceptor sites (Michel *et al.*, 1989; Ernsberger *et al.*, 1990) and has been used as a tool to identify imidazoline receptors (Ernsberger *et al.*, 1992; Reis *et al.*, 1992). In the present study, idazoxan has been demonstrated to be an effective antagonist of the $I_1$ imidazoline agonist, moxonidine, in the kidney. Lower doses of idazoxan (0.1 and 0.3 mg kg$^{-1}$) decreased the renal actions of moxonidine, whereas a higher dose failed to have any antagonistic effect. The dose of idazoxan (1.0 mg kg$^{-1}$) that failed to alter the response to moxonidine,

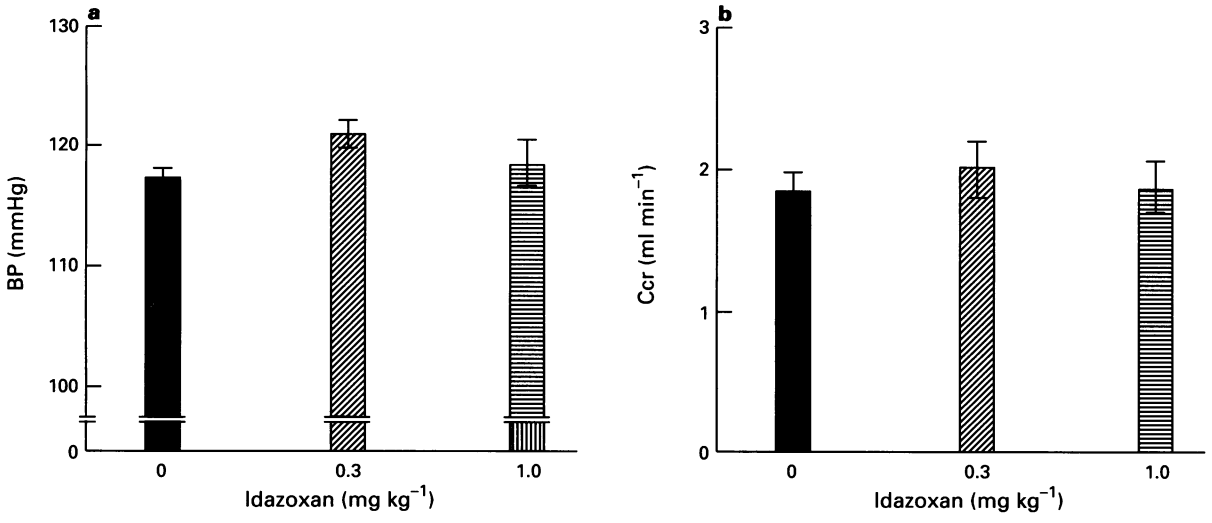


**Figure 4** The effects of increasing doses of intravenous idazoxan on mean arterial blood pressure (a) and creatinine clearance (Ccr) (b).

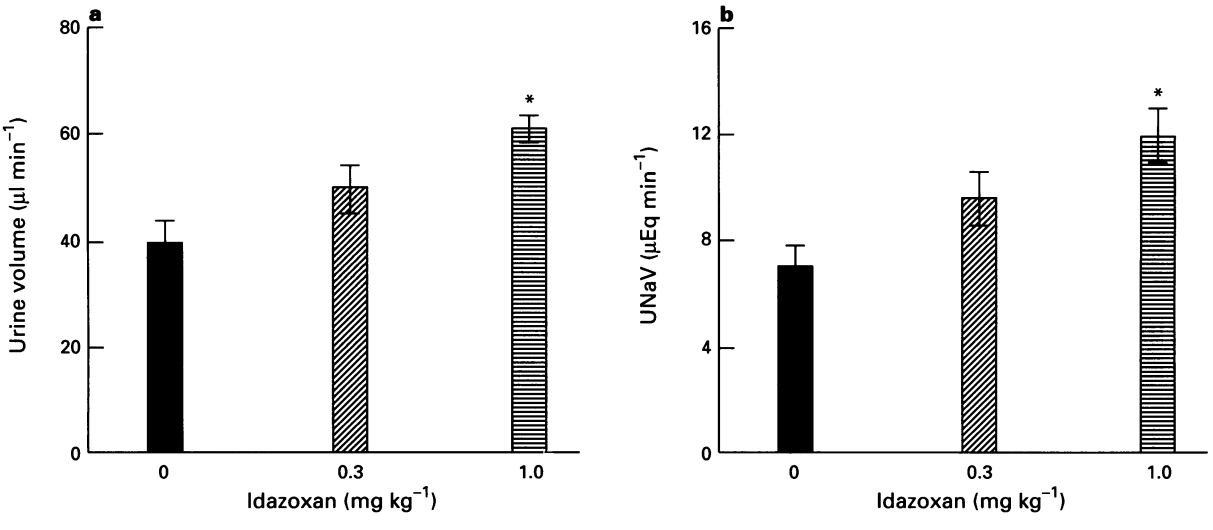


**Figure 5** The effects of increasing doses of intravenous idazoxan on urine volume and sodium excretion (UNaV). $*P<0.05$ as compared with the saline vehicle control group (solid column).

produced significant renal effects when administered alone. An apparent partial agonist action of idazoxan, or lack of specificity, with a slight increase in dosage would be consistent with the failure to alter the response to moxonidine.

The ability to subtype or define a new receptor physiologically has been facilitated by the demonstration of specific antagonism of the specific receptor site(s). This antagonistic action may take the form of either radioligand binding studies or through physiological functional studies. Previous studies have indicated how an antagonist, such as idazoxan, may be useful in the ongoing description of this novel receptor by antagonism of specific imidazoline receptor agonists such as clonidine, moxonidine and rilmenidine (Gomez *et al.*, 1991 Tibirica *et al.*, 1991; Allan *et al.*, 1993). The present study, however, indicates that inappropriate conclusions may be reached if the dose of idazoxan is slightly increased.

A classification for the imidazoline receptor subtypes has recently been proposed based on *in vitro* radioligand binding studies (Ernsberger *et al.*, 1992; Michel Ernsberger, 1992; Reis *et al.*, 1992). The $I_1$ subtype was proposed to possess a higher affinity for compounds such as clonidine and moxonidine, whereas, the $I_2$ subtype was proposed to possess a higher affinity for idazoxan. The $I_2$ subtype was further sub classified based on amiloride sensitivity ($I_{2a}$) or insensitivity ($I_{b2}$). Based on these radioligand findings *in vitro*, it would seem initially inappropriate to utilize an $I_2$-specific antagonist (idazoxan) to block the actions of an $I_1$-specific agonist (moxonidine). However, studies *in vivo* have demonstrated that idazoxan effectively antagonizes actions purported to be mediated at $I_1$-imidazoline receptors. Ernsberger *et al.* (1992) reported a significant relationship between the affinity of imidazoline agonists (clonidine, moxonidine, rilmenidine) for the $I_1$ site in the ventral lateral medulla and the blood pressure lowering potency. *In vivo* studies to date have used idazoxan to antagonize the blood pressure lowering action of $I_1$-agonists. In the nucleus reticularis lateralis, idazoxan but not yohimbine ($\alpha_2$-adrenoceptor antagonist) antagonized the blood pressure lowering and neural inhibition action of clonidine (Tibirica *et al.*, 1991). In the C1 area of the rostral ventrolateral medulla, a micro injection of idazoxan but not an $\alpha_2$-adrenoceptor antagonist, (SKF-86466), decreased the hypotensive response to the imidazoline agonist, rilmenidine (Gomez *et al.*, 1991). Idazoxan was also more effective than yohimbine in the blockade of the cardiovascular actions of rilmenidine in the rabbit (Feldman *et al.*, 1990). The renal actions of moxonidine, were blocked by specific doses of idazoxan but not rauwolscine

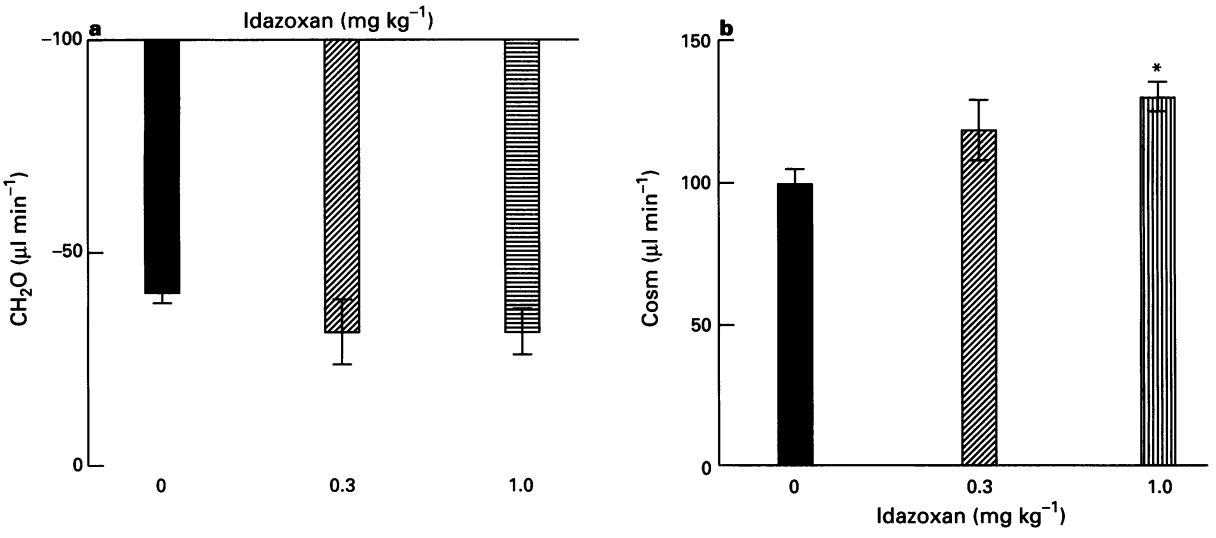


**Figure 6** The effects of increasing doses of intravenous idazoxan on free water clearance ($CH_2O$) (a) and osmolar clearance (Cosm) (b). *$P<0.05$ as compared with the saline vehicle control group (solid bar).

(Allan *et al.*, 1993). Thus, *in vivo* studies indicate that idazoxan has been effective at antagonizing effects due to stimulation of $I_1$ sites, whereas, radioligand binding studies *in vitro* indicate that, at best, idazoxan would be less effective in antagonizing effects mediated at the $I_1$ site. These studies do not indicate idazoxan is selective for the $I_1$ site as compared to the $I_2$ site, rather, they indicate that idazoxan may be useful in differentiating the imidazoline receptor mediated effects from those mediated by the $\alpha_2$-adrenoceptor only when an appropriate dose is used. Part of the difficulty has been the lack of identification of a specific $I_1$-site specific antagonist. Ernsberger *et al.* (1992) reported that efaroxan was highly specific for the $I_1$ site over the $I_2$ site. However, preliminary studies in our laboratory (unpublished observations) have found that doses of efaroxan necessary to block actions of renal $I_1$ site stimulation (Allan *et al.*, 1993) also have significant effects on blood pressure when administered alone.

Based on previous studies *in vivo*, it would appear that idazoxan has been a good tool for identifying the actions of $I_1$ site stimulation. However, the possibility of idazoxan being a partial agonist and/or lacking specificity at lower doses has not been addressed in these studies. Paciorek & Shepperson (1983) suggested that idazoxan was a partial $\alpha_1$-adrenoceptor agonist. This was based on their study of pithed female Sprague Dawley rats. They showed that idazoxan elicited a dose-related pressor response when administered in very high intravenous bolus doses (0.1–30.0 mg kg$^{-1}$) which could be blocked by the specific $\alpha_1$-adrenoceptor antagonist, prazosin. Similarly, Timmermans *et al.* (1984) found that the rise in diastolic pressure induced by intravenous idazoxan could be significantly attenuated by prazosin and/or rauwolscine. In our current experiments we noted a consistent although transient rise in mean arterial blood pressure of 5–10 mmHg lasting about 30 s following the slow intravenous bolus of the highest dose of idazoxan investigated. In our study, it was unlikely that idazoxan was a partial $\alpha_1$-adrenoceptor agonist. This would be expected to decrease urine flow rate and sodium excretion (Smyth *et al.*, 1985) and not increase these parameters as we observed.

Goldstein *et al.* (1983) using doses similar to that reported in the present study found that, whereas low doses (0.25 mg kg$^{-1}$) of RX 781094 (idazoxan) suppressed nerve firing in the locus coeruleus, a slightly higher dose (1.0 mg kg$^{-1}$) increased the firing rate. Similarly, Schwietert *et al.* (1992) found that idazoxan alone could increase the contraction amplitude of spontaneous myogenic activity of rat isolated portal vein and this effect was not related to interaction with α-adrenoceptors. In another study, idazoxan as well as a number of imidazoline receptor agonists were observed to inhibit $^{22}Na^+$ uptake with concurrent cellular alkalinization which would have been consistent with some partial agonist activity (Bidet *et al.*, 1990). Collectively, these studies indicate that idazoxan may have additional actions at doses that have been proposed as selective for the imidazoline receptor.

The increase in urine flow rate and sodium excretion may also have been mediated by $\alpha_2$-adrenoceptor antagonism since idazoxan has a fairly high affinity for this receptor (Freedman *et al.*, 1984; Dabire, 1986). However, antagonism of endogenous $\alpha_2$-adrenoceptor function in the kidney would be expected to decrease urine flow rate secondary to a decrease in free water clearance (Blandford & Smyth, 1988b; Gellai, 1990). In the present study, idazoxan increased urine flow rate secondary to an increase in osmolar clearance. Similarly, it would be unlikely that idazoxan was reversing the actions of the endogenous agonist of renal $I_1$ imidazoline receptors. A potential endogenous agonist for these receptors, agmatine, has been proposed (Li *et al.*, 1994). Preliminary experiments in our laboratory have indicated that an intrarenal infusion of agmatine increases urine flow rate modestly, secondary to an increase in osmolar clearance (data not shown). This increase in osmolar clearance is similar to the effects reported for moxonidine (Allan *et al.*, 1993). Consequently, one would anticipate that blockade of the endogenous activity of these receptors would decrease rather than increase urine flow rate as was observed in the present study.

In summary, idazoxan completely attenuated the renal actions of moxonidine at low doses. However, moderate doses of idazoxan failed to alter the response to moxonidine and in fact when administered alone produced an effect similar to that observed for moxonidine alone. These results indicate that it is necessary to use specific doses of idazoxan to achieve specificity of action. The physiological effects observed following increasing doses of idazoxan may be due to either idazoxan acting as a partial agonist at the same site and/or activity at a separate receptor site.

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