Sodium excretion following central administration of an I₁-imidazoline receptor agonist, moxonidine

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1 Previously we have shown that an intrarenal infusion of moxonidine, an I₁-imidazoline receptor agonist, resulted in a natriuresis which was inhibited by intravenous idazoxan, a selective imidazoline receptor antagonist. Therefore we examined the effects on renal function of intracerebroventricular (i.c.v.) administration of moxonidine with or without i.c.v. idazoxan.

2 Seven days after unilateral nephrectomy, Sprague-Dawley rats had i.c.v. cannulae implanted. Three days later the rats were anaesthetized (pentobarbitone), followed by cannulation of the jugular vein (fluid and drug administration), carotid artery (blood pressure) and the ureter (urine collection).

3 After a 45 min stabilization period, the effect of moxonidine was investigated by the i.c.v. administration of either isotonic saline or moxonidine (0.1, 0.3 or 1 nmol in isotonic saline) administered in 5 µl over 1 min. All doses of moxonidine resulted in an increase in urine flow with a concomitant increase in sodium excretion without affecting blood pressure. The highest dose of moxonidine (1 nmol) also increased free water clearance.

4 In a second series of experiments, the effects of idazoxan on the natriuretic response to i.c.v. moxonidine were determined. Moxonidine (0.3 nmol) again increased sodium and water excretion as compared to the i.c.v. saline control animals. Pretreatment with i.c.v. idazoxan (0.3 nmol), at a dose which alone failed to alter sodium and water excretion, completely attenuated the renal response to moxonidine. These results are consistent with central I₁-imidazoline receptors mediating a moxonidine-induced increase in sodium and water excretion at doses that do not alter blood pressure.

Keywords: Natriuresis; intracerebroventricular; antihypertensive

Introduction

The central nervous system via the sympathetic nervous system has been shown to have an important role in the control and modulation of renal salt and water handling (Kopp & DiBona, 1992). A number of the physiological responses previously attributed to the α₂-adrenoceptor may in fact be mediated through activation of imidazoline receptors (IRs). Several authors have identified IRs in the central nervous system (Ernsberger et al., 1987; Bricca et al., 1989). Two different IR subtypes have been proposed, I₁ and I₂ (Ernsberger, 1992). A clear physiological role involving the interrelationship between central imidazoline receptors and the kidney has yet to be elucidated.

Previous work demonstrated that, with lower doses of clonidine the increase in urine flow rate was primarily due to an increase in free water, while higher doses resulted in an increase in sodium excretion as reflected in osmolar clearance (Blandford & Smyth, 1988). The increase in free water clearance following intrarenal infusions of the α₂-adrenoceptor agonist, clonidine, was secondary to antagonism of vasopressin (Blandford & Smyth, 1990). In our laboratory, using different purported α₂-adrenoceptor agonists, we have demonstrated a dissociation between two physiological functions in the kidney (Smyth et al., 1992). These studies suggested two distinct receptors and the increase in sodium excretion with higher doses of clonidine could be due to activation of the IR. Subsequently, our laboratory demonstrated that the intrarenal administration of moxonidine, a selective I₁-IR agonist, increased sodium and osmolar clearance, an effect that was blocked by intravenous idazoxan, an IR antagonist (Allan et al., 1993). This suggested a physiological role for the IR in the kidney.

Central administration of compounds with high affinity for the imidazoline receptor result in haemodynamic changes (Ernsberger et al., 1988; 1990). In the current study, we examined changes in renal function following the intracerebroventricular (i.c.v.) administration of the selective IR agonist, moxonidine, using doses that were less than are necessary to result in haemodynamic changes. By using clearance techniques, we were able to dissociate water handling from osmolar or solute handling by the kidney. This allowed us to draw conclusions about the effect different i.c.v. doses of moxonidine had on these parameters. Central administration of idazoxan, a selective IR antagonist, was used to inhibit i.c.v. moxonidine directly. Thus, the postulate that the changes in sodium excretion previously reported with central administration of purported α₂-adrenoceptor agonists could be through the activation of the IR, was examined in the current study.

Methods

Experimental preparation

Male Sprague-Dawley rats (200–250 g) underwent unilateral nephrectomy (ether anaesthesia) 1 week prior to placement of an i.c.v. cannula. Animals were housed at 23°C with a 12/12 h light/dark cycle. On the day of the experiment the rats were anaesthetized with pentobarbitone (BDH Chemicals Ltd., Poole, England, 50 mg kg⁻¹). The animals were placed on a heating blanket which was thermostatically controlled to maintain body temperature at 37.5°C. A tracheotomy was performed and the animals were allowed to breathe spontaneously. The carotid artery was cannulated for measurement of blood pressure, heart rate and obtaining a blood sample at the end of the experiment. The jugular vein was cannulated for administration of drugs. Blood pressure was monitored with a Statham pressure transducer (model P23Dc) connected to a Grass polygraph model V. A modest
diuresis was maintained throughout the experiment by administration of isotonic saline (97 μl min⁻¹). The moxonidine and idazoxan, which were dissolved in isotonic saline, were given centrally in a volume of 5 μl over 1 min with a 10 μl Hamilton syringe. The remaining kidney was exposed by a flank incision and the ureter was cannulated with PE-50 for collection of urine. The urine volumes were determined gravimetrically.

Intracerebroventricular cannula

The method used to implant the cannulae was the same as previously described (Penner et al., 1990). Briefly, the rats were anaesthetized by an intraperitoneal injection of 50 mg kg⁻¹ pentobarbitone. The cannula was a modified 23 gauge needle with a solid obturator, threaded through the lumen and extending 0.5 mm beyond the tip of the needle. The head of the rat was placed in a stereotaxic apparatus (model 900, David Kopf, Tujunga, CA, U.S.A.). The coordinates for implantation of the cannula in relation to the skull were 0.3 mm posterior to the bregma, 1.4 mm lateral to the midline and 3.5 mm below the surface of the skull. The cannula was anchored to the skull with three stainless steel jewellry screws in an assemblage of acrylic cement. At the time of the experiment the obturator was removed. A 31 gauge injector tube was inserted which extended 1.0 mm below the end of the guide tube (23 gauge needle) and consequently entered the ventricle. The injector tube was then connected to a 10 μl Hamilton syringe for administration of drug or vehicle. Verification of the cannula location in the cerebral ventricle was performed by injection of dye through the cannula at the end of the experiment, and examination of postmortem brain sections.

Intracerebroventricular moxonidine with or without idazoxan

Three days after the placement of the i.c.v. cannulae the experimental procedure, as outlined above, was performed.

Figure 1 The effects of increasing dose of i.c.v. moxonidine on blood pressure (a), heart rate (b) and creatinine clearance (c) in rats (6 per group). The first time period represents a pretreatment 15 min control urine collection. Time periods 2 to 5 represent 15 min urine collections in the presence of saline vehicle or increasing doses of moxonidine. The different i.c.v. infusions are illustrated as follows: (●) saline control; (○) moxonidine 0.1 nmol; (△) moxonidine 0.3 nmol; (□) moxonidine 1 nmol. The data are presented as the mean ± s.e.mean. *P < 0.05.

Figure 2 The effects of increasing dose of i.c.v. moxonidine on urine flow (a) and sodium excretion (UNaV) (b) in rats (6 per group). The time periods are as described in Figure 1. The different i.c.v. infusions are illustrated as follows: (●) saline control; (○) moxonidine 0.1 nmol; (△) moxonidine 0.3 nmol; (□) moxonidine 1 nmol. The data are presented as the mean ± s.e.mean. *P < 0.05.
Following the surgical preparation and a 45 min stabilization period, a 15 min control urine collection was obtained. In the first series of experiments, immediately following this control urine collection, i.c.v. isotonic saline or moxonidine (0.1, 0.3 or 1 nmol) was administered. This was followed by four further 15 min urine collections. In a second series of experiments, the effects of the IR antagonist, idazoxan on the response to moxonidine was assessed. In these experiments, following the control urine collection, idazoxan (0.3 nmol) or isotonic saline was administered i.c.v. followed by moxonidine (0.3 nmol) or isotonic saline. Ten minutes later four post-treatment urine collections of 15 min each were obtained. The four treatment groups for the antagonist study were: saline controls, saline followed by moxonidine, idazoxan followed by saline, and idazoxan followed by moxonidine. Each study was carried out in separate animals. At the completion of the experiment a blood sample was obtained and the plasma frozen for later analysis. Urine and plasma creatinine levels were measured with a Beckman Creatinine 2 Analyzer; sodium was measured with a Beckman Klama Flame Photometer; and osmolality was assessed with a Precision System Micro Osmometer.

Statistical analysis

The data are presented as the mean ± standard error of the mean. Each group contained six animals. Repeated measures of analysis of variance (ANOVA) utilizing SAS System Version 6.07 was used to assess the data. Where significant differences were found with the ANOVA the interactions were analyzed using the Least Squares Means Difference Test. A P value of ≤ 0.05 was used for significance and is denoted by * in the figures.

Drugs

Moxonidine was supplied by Berisdorf, AG, Hamburg, FRG and idazoxan was obtained from Research Biochemicals, Inc., Natick, MA.

Results

Dose-response to intracerebroventricular moxonidine

At all doses investigated in the present study, i.c.v. administration of moxonidine had minimal effects on blood pressure and heart rate. The lowest dose of i.c.v. moxonidine investigated (0.1 nmol) produced a transient increase in blood pressure during the first collection immediately following i.c.v. administration. This dose was also associated with a small but significant increase in creatinine clearance (Figure 1). Urine flow rate was increased by all doses of moxonidine. Concomitant with the increase in urine flow was an increase in sodium excretion (Figure 2). Similarly, osmolar clearance was also increased with all three doses of i.c.v. moxonidine (Figure 3). The lowest dose of moxonidine (0.1 nmol) produced the greatest increase in osmolar clearance, which may have been secondary to the increase in creatinine clearance observed with this dose. Free water clearance was decreased by 0.1 nmol of moxonidine, reflecting the greater increase in osmolar clearance for a given urine flow rate. At the highest dose of moxonidine (1 nmol) investigated, free water clearance was increased.

Intracerebroventricular moxonidine and idazoxan

In the second series of experiments, i.c.v. moxonidine alone, i.c.v. idazoxan alone, or i.c.v. moxonidine and idazoxan failed to alter blood pressure, heart rate and creatinine clearance as compared to the animals receiving the saline control vehicle (Figure 4). Idazoxan (0.3 nmol) alone slightly decreased the urine flow rate but did not alter the sodium excretion (Figure 5). Moxonidine (0.3 nmol), as expected from the previous series of animals, increased both the urine flow rate and the sodium excretion (Figure 5). Administration of idazoxan prior to the moxonidine resulted in a complete attenuation of the diuretic and natriuretic response to moxonidine (Figure 5). Similarly, the increase in osmolar clearance observed following moxonidine was antagonized by the concomitant administration of idazoxan (Figure 6). At the doses chosen in this set of experiments no changes in free water clearance were observed.

Discussion

Initially, clonidine was reported to be a specific α2-adrenoceptor agonist, and consequently, the blood pressure lowering action of this drug was postulated to be mediated by activation of central α2-adrenoceptors (Kobinger, 1978). Studies by Bousquet et al. (1984) challenged this concept with the examination of the cardiovascular effects of different α2-adrenoceptor agonists given centrally. In this study by Bousquet et al. in 1984, the agonists were not categorized according to previously reported specificity for α2- and α1- adrenoceptors, but on structure – imidazoline (i.e. clonidine) versus phenylethylamine (catecholamines). The blood pressure lowering actions of these various agonists were assessed in the nucleus reticularis lateralis. Phenylethylamine-based compounds failed to alter blood pressure, whereas imidazoline-based agonists, whether previously reported to be α2- (i.e. cirazoline) or α1- (i.e. clonidine) adrenoceptor-specific, pro-
duced significant decreases in blood pressure. This indicated that this effect was not mediated by α₂-adrenoceptors per se but rather by binding sites which had a higher affinity for imidazoline-based molecules (imidazoline-sites). Paralleling these findings were radioligand binding studies in the central nervous system which demonstrated two populations of receptors, one binding α₂-adrenoceptor compounds, while the second site preferentially bound imidazoline based compounds (Ernsberger et al., 1987; Bricca et al., 1989). Subsequently, the endogenous clonidine-displacing substance was shown to bind to these same receptors with a high affinity and low capacity, thus demonstrating a possible endogenous agonist for this novel receptor (Parini et al., 1989). These studies indicated a potential role of the imidazoline receptor in the regulation of blood pressure.

A number of studies have demonstrated that clonidine and structurally related compounds lower blood pressure through stimulation of imidazoline receptors located centrally (Feldman et al., 1990; Tibirica et al., 1991; Gomez et al., 1991). Clonidine, through what now appears to be imidazoline receptors located in the ventrolateral medulla, has been found to lower blood pressure in a number of different species (Gillis et al., 1985; Sinha et al., 1985; McAuley et al., 1988; Feldman et al., 1990). This blood pressure lowering has been attributed to a suppression of catecholaminergic neurones located centrally which in turn would lower peripheral sympathetic nerve activity (Tibirica et al., 1989; 1992). The central nervous system has also been shown to modulate renal sodium excretion through changes in renal nerve activity irrespective of changes in blood pressure (Koepeke et al., 1987). This suggested that stimulation of imidazoline receptors centrally, in addition to having a vasodepressor action, could also function to regulate water and sodium excretion.

In the current study, i.c.v. administration of moxonidine resulted in an increase in urine flow rate and sodium excretion with all doses utilized. This was observed at doses of moxonidine which failed to alter blood pressure, creatinine clearance or heart rate suggesting that the i.c.v. moxonidine

Figure 4 The effects of i.c.v. moxonidine (0.3 nmol) on blood pressure (a), heart rate (b) and creatinine clearance (c) in rats (6 per group) in the presence and absence of idazoxan. The time periods are as described in Figure 1. The different i.c.v. infusions are illustrated as follows: (●) saline control; (◇) moxonidine 0.3 nmol and saline; (□) moxonidine 0.3 nmol and idazoxan 0.3 nmol; (◆) saline and idazoxan 0.3 nmol. The data are presented as the mean ± s.e.mean. *P<0.05.

Figure 5 The effects of i.c.v. moxonidine (0.3 nmol) on urine flow rate (a) and sodium excretion (UNaV) (b) in rats (6 per group) in the presence and absence of idazoxan (0.3 nmol). The time periods are as described in Figure 1. The different i.c.v. infusions are illustrated as follows: (●) saline control; (◇) moxonidine 0.3 nmol and saline; (□) moxonidine 0.3 nmol and idazoxan 0.3 nmol; (◆) saline and idazoxan 0.3 nmol. The data are presented as the mean ± s.e.mean. *P<0.05.
was specifically altering renal function, independent of cardiovascular function. The doses of i.c.v. moxonidine were chosen based on preliminary studies in which lower doses were without effect, while higher doses than used in the current study resulted in haemodynamic changes. The doses in the present study of 0.1 to 1 nmol were considerably lower than the total dose of moxonidine used by Allan et al. (1993): 1 to 10 nmol kg$^{-1}$ min$^{-1}$ continuously for 1 h. Thus, it is unlikely that the observed changes following i.c.v. moxonidine are due to a direct peripheral effect. As previously observed with the vasodilator action of imidazoline receptor agonists (Feldman et al., 1990; Tibirica et al., 1991; Gomez et al., 1991), in the current study changes in renal function observed with i.c.v. moxonidine were antagonized with i.c.v. idazoxan. This is consistent with the natriuresis following central administration of moxonidine being secondary to activation of the imidazoline receptors. The highest dose used also increased free water clearance. The mechanism for this observation was unclear, but may be related to this higher dose of moxonidine stimulating $\alpha_2$-adrenoceptors. This, in turn, may alter the central release of vasopressin or the stimulation of peripheral $\alpha_2$-adrenoceptors which antagonize the renal actions of vasopressin (Blandford & Smyth, 1990). Further studies will be necessary to elucidate both the mechanism for this finding, as well as, the respective role of the central versus the peripheral activation of the imidazoline receptor in the regulation of sodium excretion.

More recently, radioligand binding studies have identified imidazoline-receptors in the kidney (Bidet et al., 1990). In an initial study, we determined the effect of three different purposed $\alpha_2$-adrenoceptor agonists, clonidine, UK 14,304 and 2.6 dimethyl clonidine, on free water clearance and osmolar clearance. The results for free water clearance demonstrated a rank order of potency of clonidine >> UK 14304 >> 2.6 dimethyl clonidine, which was reversed for osmolar clearance (Smyth et al., 1992). As these were distinct physiological effects, these findings were consistent with two distinct receptors and/or sites. Additional studies, using the selective $I_1$ imidazoline receptor agonist, moxonidine, infused directly into the renal artery, demonstrated a dose-related natriuresis at doses of 1, 3 and 10 nmol kg$^{-1}$ min$^{-1}$ (Allan et al., 1993). The specific imidazoline receptor antagonist, idazoxan, inhibited the response but rauwolscine (specific $\alpha_2$-adrenoceptor antagonist) was without effect. This was consistent with the increase in sodium being secondary to activation of renal imidazoline receptors. At present, it is not clear as to whether central and peripheral $I_1$-imidazoline receptors function exclusively of each other to alter sodium and water excretion. Conceivably, centrally administered moxonidine may have stimulated renal receptors, or peripherally administered moxonidine may have altered function at central sites to alter renal function. Further studies will be required to determine the specific sites of action.

Previous studies have clearly demonstrated a vasodepressor action of centrally administered imidazoline receptor agonists. In the current study, we have demonstrated that moxonidine, a specific $I_1$-imidazoline receptor agonist, administered i.c.v. produced an increase in urine flow rate and sodium excretion at levels which appeared to have negligible cardiovascular actions. As this appeared at doses lower than those required to alter blood pressure acutely, this centrally mediated natriuresis may represent an additional mechanism by which these agonists function as antihypertensives.

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References


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