Biochimica et Biophysica Acta, 1024 (1990) 173–178
Elsevier

BBAMEM 74836

Effect of imidazolines on Na^+ transport and intracellular pH in renal proximal tubule cells

Michel Bidet [1], Philippe Poujeol [1] and Angelo Parini [2]

[1] *Département de Biologie, Service de Biologie Cellulaire, Centre d'Etudes Nucléaires de Saclay, Gif-sur-Yvette, and* [2] *CNRS UA 318, Laboratoire de Pharmacologie, Faculté de Médecine Necker-Enfants Malades, Paris (France)*

(Received 19 July 1989)
(Revised manuscript received 28 December 1989)

Key words: Proximal tubule; Imidazoline derivative; Sodium ion influx; pH, intracellular; (Rabbit kidney)

Recently, we characterized an imidazoline-guanidinium receptive site (IGRS) in the renal proximal tubule of rabbit kidney. Although recognized by a series of imidazoline and guanidinium alpha-2 adrenergic compounds, IGRS is insensitive to catecholamines and can be physically separated from alpha-2 adrenergic receptors after solubilization. In the present study, we investigated the effect of imidazoline derivatives on $^{22}Na^+$ uptake and intracellular pH in isolated cells from rabbit renal proximal tubule. After 5 min of preincubation, idazoxan inhibited the total $^{22}Na^+$ influx (−30%) in a dose-dependent manner, with a maximum effect at 10^{-5} M. The effect of idazoxan was not competitive as shown by the decrease of the maximal velocity of $^{22}Na^+$ entry (control: 3.80 ± 0.42; idazoxan 10^{-5} M: 3.23 ± 0.33 nmol/30 s per mg protein, $P < 0.01$). A series of imidazoline derivatives inhibited $^{22}Na^+$ entry with an order of potency similar to that previously found for inhibition of [^{3}H]idazoxan binding to IGRS (cirazoline > idazoxan > UK 14304 > rilmenidine ≫ cimetidine). The inhibition of $^{22}Na^+$ uptake by these compounds does not appear to be related to interaction with alpha-adrenergic receptors since it was observed in the presence of saturating concentrations of the adrenergic antagonists rauwolscine (alpha-2) or prazosin (alpha-1). When tested on the regulation of intracellular pH by fluorimetric techniques, 10^{-5} M cirazoline or idazoxan inhibited by 20% the velocity of the sodium-dependent H^+ efflux in acidified cells ($P < 0.02$). The concomitant inhibition of $^{22}Na^+$ entry and of cell realkalinization suggests that imidazoline derivatives inhibit Na^+/H^+-exchanger. This effect could be mediated via the renal IGRS and intracellular second messengers that are not yet known.

Introduction

Since the first demonstrations that catecholamines modulate sodium reabsorption in the renal proximal tubule [1,2], many studies attempted to characterize the adrenergic receptor subtype responsible for this activity. Although a large body of evidence supports the hypothesis that alpha-adrenergic receptors are involved in the tubular sodium handling, different studies suggested that either alpha-1 [3,4] or alpha-2 [5,6] receptors can modulate sodium reabsorption in the renal proximal tubule. This discrepancy in the results could be ascribed to the different animal models employed as well as to the synthetic molecules used to characterize the receptor subclass. Indeed, it has been recently shown that prazosin, a highly selective alpha-1 antagonist, also interacts with high affinity with renal alpha-2 receptors in rat [7].

In some studies, the role of alpha-2 adrenergic receptors in sodium reabsorption has been also investigated using imidazolines (idazoxan, UK 14304) [8] or guanidinium (guanabenz) analogs [8,9]. However, we have recently found that these molecules label not only alpha-2 but also an imidazoline-guanidinium receptive site (IGRS) [10,11]. Although this binding site shares with alpha-2 receptors a high affinity for imidazolines, it is completely insensitive to catecholamines and can be physically separated with alpha-2 receptors after solubilization [12]. As shown by competition binding studies, IGRS is not recognized by endogenous ligands for known receptor-proteins but interacts with an endogenous clonidine-displacing substance (CDS) partially purified from calf brain [13]. Even if this substance also interacts with alpha-2 adrenergic receptors [13], it induces peripheral effects [14,15] and elicits centrally-mediated changes of systemic blood pressure [16] by

Correspondence: M. Bidet, DB/SBCe, Centre d'Etudes Nucléaires de Saclay, 91191 Gif-sur-Yvette, Cedex, France.

mechanisms distinct from the stimulation of alpha-2 adrenergic receptors.

In order to characterize the functional activity elicited by the stimulation of IGRS, we investigated the effects of different imidazoline derivatives on sodium transport and intracellular pH regulation in isolated proximal cells from rabbit kidney.

Material and Methods

Isolated proximal cell preparation. For each experiment, one adult male New Zealand white rabbit (1.5–2.0 kg body weight) was used. All animals were fed a standard diet and had free access to tap water. For cell preparations, kidneys were removed from animals sacrified by 5 ml pentobarbital sodium and 2.500 U heparin (Roussel, France) injected through the vein of the ear. Experiments were performed on isolated proximal cells from rabbit kidney prepared as previously described by Poujeol and Vandewalle [17]. Briefly, kidneys were perfused with RPMI 1640 medium (GIBCO, Grand Island, NY) devoid of sodium bicarbonate and buffered with 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes) at pH 7.4. Slices from the superficial cortex were then passed through a tissue press and successively filtered over 100-, 40- and 20-μm nylon meshes. This procedure yielded from $100 \cdot 10^6$ to $200 \cdot 10^6$ cells. The total number of cells was estimated by microscopic observation on 20 μl of cell suspension diluted in 0.5% eosin solution; protein content was measured according to the Bradford method [18] (Bio-Rad protein assay, Bio-Rad Laboratories, München, F.R.G.) using bovine serum albumin (BSA) as reference. The cells were used at a final concentration of $50 \cdot 10^6$/ml in RPMI medium.

^{22}Na uptake in isolated cells. Measurements of ^{22}Na uptake by the isolated cells were performed at 37°C according to the centrifugation method previously described [17]. After preparation, isolated cells were rinsed three times in a mannitol buffer containing (in mM) 280 mannitol, 5 KCl, 1 $CaCl_2$, 0.4 $MgSO_4$, 2 glutamine, 20 Hepes (pH 7.4), and kept 30 min at 37°C in order to ensure sodium depletion [17]. Aliquots of 100 μl of cell suspension ($5 \cdot 10^6$) were equilibrated at 37°C in presence of $2 \cdot 10^{-4}$ M ouabain to block the Na^+,K^+ pump. The uptake was initiated by the addition of 100 μl mannitol buffer containing 3 mM ^{22}Na (Amersham, U.K.) and terminated by removing 180 μl from the suspension and diluting this sample in 1 ml of ice-cold mannitol stop solution. The cells were centrifuged for 30 s in a Beckman centrifuge, and the pellet was washed twice. The radioactivity was determined by scintillation counting (Intertechnique SL 4000) after dissolving the pelleted cells in 500 μl 1 M NaOH. All incubations were carried out in triplicate. The extracellular water space was measured in each experiment by using [^{3}H]sorbitol, and Na^+ data were corrected for this factor. The part of the extracellular volume in the pellet represented 20–30% of total water volume.

Intracellular pH and proton efflux measurements. Measurements of pH_i were performed fluorimetrically using 2,7-biscarboxyethyl-5(6)-carboxyfluorescein (BCECF)-loaded cells as previously described [19]. The isolated proximal tubular cells ($50 \cdot 10^6$/ml) were incubated in RPMI medium containing 4 μg/ml BCECF/AM (Calbiochem Corp., La Jolla, CA) for 30 min at 37°C. Just after the incubation period, the cells were rinsed twice in fresh RPMI. Then aliquots of cells were resuspended in tetramethylammonium (TMA) medium (in mM): 140 TMA-Cl, 5 KCl, 1 $CaCl_2$, 0.4 $MgSO_4$, 2 glutamine, 10 glucose, 20 Hepes (pH 7.4) and acid loaded for 10 min by the nigericin technique [19]. Acid load was followed by centrifugation for removal of albumin and nigericin. Excitation and emission wavelengths were 500 and 530 nM, and 5- and 10-nm slits were used, respectively. In all experiments, pH_i measurements were performed at room temperature in 3-ml quartz cuvettes containing $5 \cdot 10^6$ proximal cells suspended in 2 ml of the appropriate buffer. During the measurement, cells were stirred with a magnetic stirrer. At the end of each individual fluorescence recording, the buffering capacity was determined by the NH_4^+ technique [20]. The fluorescence signal related to intracellular pH changes were calibrated after cells lysis using Triton X-100 [21]. Variations of pH_e between 7.4 and 6.3 were obtained by successive additions of 1 N 2-(*N*-morpholino)ethanesulfonic acid. The proton efflux as a function of time was calculated as follows: H^+ efflux = buffering power $\times$ pH_i (in $mmol \cdot l^{-1} \cdot pH\ unit^{-1}$). In all experimental conditions, the fluorescence was continuously recorded by using a Perkin-Elmer fluorimeter and the pH_i was measured using the first 30 s in order to calculate the initial rate of proton efflux.

Statistical analysis. Values reported in the text and tables are the means ± S.E. Student's *t*-test was used for statistical analysis.

Results

Effect of imidazolines on ^{22}Na uptake in isolated proximal cells

The effect of imidazoline derivatives in ^{22}Na influx was measured in sodium-depleted cells. These cells retain receptor mediated functions such a the PTH-sensitive adenylate cyclase activation and the modulation of the Na^+/H^+ antiporter and Na^+/phosphate cotransport by glucorticoids [17,22].

The uptake of 1.5 mM ^{22}Na was linear for one minute as previously described [19]. Therefore, in all subsequent experiments, the measurements were performed at 30 s to estimate the effect of imidazoline derivatives on the initial rate of Na^+ entry. Fig. 1 shows

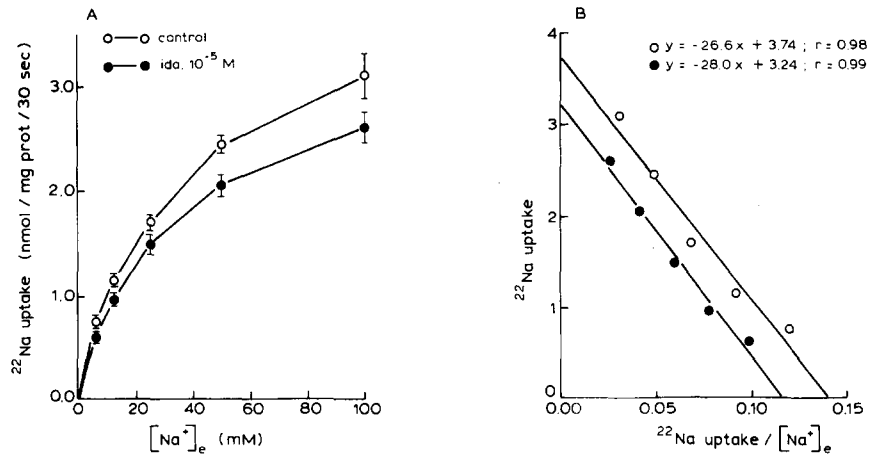

Fig. 1. ^{22}Na uptake by isolated proximal cells: effect of idazoxan at different external Na^+ concentration ($[Na^+]_e$). ^{22}Na uptake was measured on $5 \cdot 10^6$ cells suspended in mannitol buffer after 30 s at 37 °C. Each point represents the mean ± S.E. of five experiments. (A) effect of idazoxan (10^{-5} M) at different external sodium concentrations (6.25–100 mM) (B) Eadie-Hofstee linearization of the data of (A). Control (○), idazoxan (●).

the uptake of ^{22}Na as a function of different concentrations of extracellular sodium in control cells and in cells preincubated with 10^{-5} M idazoxan. The ^{22}Na influx was dependent on the extracellular sodium concentration, reaching a maximum between 50 and 100 mM. Idazoxan decreased the initial rate of sodium entry at all sodium concentrations (6–100 mM) (Fig. 1A). The Eadie-Hofstee plot (Fig. 1B) of the data indicates that idazoxan inhibited sodium-uptake by a non-competitive mechanism since the V_{max} (control: 3.80 ± 0.42 nmol/30 s per mg protein; idazoxan: 3.23 ± 0.33 nmol/30 s per mg protein, $P < 0.01$) but not the K_m (control: 27.5 ± 4.8 mM; idazoxan: 28.0 ± 4.6 mM, n.s.) was modified in the presence of idazoxan.

The effect of increasing concentrations of idazoxan is shown in Fig. 2A. The inhibition of Na^+ entry was dose-dependent reaching the maximum at 10^{-5} M. Similar results were obtained using cirazoline, another compound exhibiting a high affinity for imidazoline receptor [10] (Fig. 2B). The effect of cirazoline was unrelated to the stimulation of tubular alpha-1 receptors since it was not affected by 10^{-6} M prazosin (data not shown).

Table I shows the effect of idazoxan on $^{22}Na^+$ uptake tested in the presence and absence of amiloride and at acid extracellular pH. In the absence of idazoxan, the addition of 1 mM amiloride to the incubation medium induced 52% inhibition of the total ^{22}Na influx which is due to the inhibition of the Na^+/H^+ antiporter [19]. A similar decrease in $^{22}Na^+$ influx was observed after inhibition of Na^+/H^+ antiporter by lowering extracellular pH. Both in the presence of amiloride or at acid pH_e, the addition of 10^{-7} M idazoxan to the incubation medium did not further inhibit the ^{22}Na influx. The lack of idazoxan effect at acid pH_e is not due to the impairment of ligand–IGRS interaction since the binding of [^{3}H]idazoxan to puri-

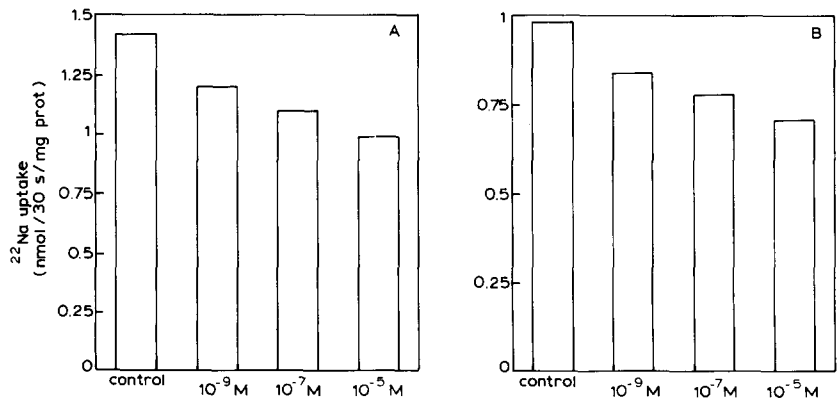

Fig. 2. Effect of different concentrations of idazoxan (A) and cirazoline (B) on $^{22}Na^+$ uptake. Isolated cells were first incubated for 5 min at room temperature in the absence (control) or in the presence of imidazoline derivatives (10^{-9} M, 10^{-7} M and 10^{-5} M). The samples were equilibrated at 37 °C and then uptake of 1.5 mM [^{22}Na]sodium chloride was determined after 30 s in mannitol buffer. The plots are representative of 2–4 experiments.

TABLE I

Effect of amiloride and external pH on the inhibition of ^{22}Na uptake by idazoxan

Isolated cells were first incubated for 5 min at room temperature in the absence (control) or in the presence of 10^{-7} M idazoxan. Uptake of 1.5 mM [^{22}Na]sodium chloride was determined at 37°C after 30 s in mannitol solution. n.s., not significant.

	n	^{22}Na influx (nmol/30 s per mg protein)		
		control	*P*	idazoxan
pH_e 7.40	7	0.78 ± 0.12	< 0.01	0.64 ± 0.10
0.5 mM amiloride	5	0.37 ± 0.05	n.s.	0.37 ± 0.05
pH_e 6.30	3	0.33 ± 0.07	n.s.	0.34 ± 0.07

fied basolateral membranes from rabbit kidney was similar at pH 6.3 and 7.4 (Coupry et al., unpublished results).

As observed for idazoxan and cirazoline, rilmenidine and UK 14304, two compounds having a high affinity for imidazoline receptor [10,11], inhibited the ^{22}Na influx by 8.7 and 15.8%, respectively (Fig. 3). The effect of these two compounds was tested in the presence of 10^{-5} M rauwolscine in order to saturate alpha-2 receptors. In contrast to that observed with the other imidazolines, 10^{-5} M cimetidine did not significantly modify the ^{22}Na uptake.

The order of potency for the inhibition of ^{22}Na influx by imidazolines was cirazoline > idazoxan > UK 14304 > rilmenidine ≫ cimetidine. A similar order of potency has been observed for inhibition of [^{3}H]idazoxan binding to basolateral membranes from rabbit renal proximal tubule [10,11,29].

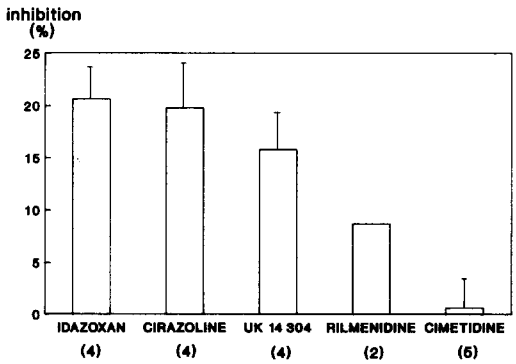

Fig. 3. Effect of imidazoline derivatives on the ^{22}Na uptake by isolated proximal cells. Isolated cells were first incubated for 5 min at room temperature in the absence or in the presence of imidazoline derivatives (10^{-7} M). For the α_2-adrenergic agonists, UK 14304 and rilmenidine, 10^{-5} M of yohimbine was added to the incubation medium. Uptake of 1.5 mM [^{22}Na]sodium chloride was determined after 30 s at 37°C in mannitol buffer. The values represent the percentage of inhibition of the total ^{22}Na influx and are means ± S.E. of (*n*) experiments.

TABLE II

Effect of imidazoline derivatives on the Na^+-induced proton efflux

The data show the mean ± S.E. for *n* cell preparations. Isolated cells were first incubated for 5 min in the absence (control) or in the presence of idazoxan or cirazoline at room temperature. pH_i were determined after acidification by the nigericin technique. Alkalinization of the cells was induced by 74 mM NaCl. H^+ efflux was calculated from fluorimetric traces. * $P < 0.02$; ** $P < 0.05$ significantly different from control H^+ efflux.

		n	pH_i after acid load	H^+ efflux ($mmol \cdot l^{-1} \cdot min^{-1}$)
Control		6	6.49 ± 0.04	19.01 ± 0.65
Idazoxan	10^{-5} M	4	6.55 ± 0.03	16.60 ± 0.27 **
	10^{-7} M	3	6.58 ± 0.05	17.06 ± 0.54
Cirazoline	10^{-5} M	4	6.52 ± 0.06	15.35 ± 0.71 *
	10^{-7} M	3	6.52 ± 0.07	16.64 ± 0.90

Effect of imidazoline derivatives on intracellular pH

The effect of imidazoline derivatives was first investigated at physiological values of intra- and extracellular pH (pH_i, 7.13; pH_e, 7.40). The addition of 10^{-5} M cirazoline or idazoxan to the cell suspension did modify the intracellular pH for a time of incubation up to 10 min (data not shown). In order to investigate the effect of imidazoline derivatives on the activated Na^+/H^+ antiporter, the cells were acidified as described in the Methods section. After acidification, preincubation of the cells with either 10^{-5} M cirazoline or idazoxan inhibited the Na^+-dependent realkalinization by 20%. This effect was not related to a non-specific effect of imidazoline derivatives on cell acidification since the acid pH in the presence of cirazoline or idazoxan was not different from control values (Table II).

The inhibition of cell alkalinization was also observed at lower concentrations of cirazoline and idazoxan (10^{-7} M). However, the small effects observed at these concentration were not statistically significant.

Discussion

Previous pharmacological and biochemical studies have identified an imidazoline-guanidinium receptive site (IGRS) that, in basolateral membranes of the proximal tubule cells, is physically distinct from alpha-2 adrenergic receptors [10,12]. At least three different observations suggest that IGRS might be involved in the regulation of sodium transport across the plasma membrane: (1) ^{22}Na influx in rat proximal tubule segments is differently regulated by phenylethylamine and imidazoline/guanidinium alpha-2 agonists [23], (2) amiloride and derivatives, usually considered as specific inhibitors of sodium transport, display a high affinity for the imidazoline-guanidinium site [12,24,25,29] and (3) the effect of guanabenz or clonidine on renal sodium

handling [23,26] cannot be explained by the previously described increase in ^{22}Na influx in tubular isolated cells following alpha-2 receptor stimulation [27].

As shown in Fig. 3, a series of imidazoline derivatives inhibited ^{22}Na influx in isolated cells. Such an effect has been previously reported in human placenta where clonidine, an imidazoline alpha-2 receptor agonist, and cimetidine, an imidazoline H_2 receptor antagonist, inhibit the Na^+/H^+ antiporter [28]. The effect of these molecules occurred at high concentrations (K_i in the micromolar range) and was competitive with respect to Na^+ ions, suggesting a direct interaction of clonidine and cimetidine with the Na^+/H^+ exchange protein ('amiloride-like' effect).

In our study, the effect of imidazoline compounds in cellular ^{22}Na influx appears to be different from that described in human placenta. Indeed, in renal proximal tubule cells, ^{22}Na uptake was inhibited by lower concentrations of imidazoline derivatives and was not affected by cimetidine. Moreover, the effect of imidazoline drugs was not competitive, as shown by the change in maximal velocity of ^{22}Na uptake in presence of idazoxan. These data suggest that imidazoline derivatives modulate sodium influx in proximal tubule cells by a mechanism clearly distinct from the direct inhibition of the Na^+/H^+ antiporter by amiloride.

The non-competitivity of imidazoline effects suggests that inhibition of sodium transport could be mediated by a membrane receptor. The localization in the basolateral side of the renal proximal tubule [10] and the high affinity for imidazoline drugs make alpha-2 adrenergic receptors the potential target for imidazoline derivatives for inhibition of $^{22}Na^+$ influx. However, this possibility appears unlikely for the following reasons: (1) the inhibition of ^{22}Na uptake was observed not only with alpha-2 adrenergic agonists but also with the alpha-1 agonist cirazoline and the alpha-2 antagonist idazoxan; (2) previous studies indicate that stimulation of tubular alpha-2 receptors enhances rather than decreases the sodium influx [23,27]; (3) the effect of imidazoline derivatives was observed in the presence of saturating concentrations of rauwolscine (10^{-5} M) to 'mask' alpha-2 adrenergic receptors.

The recent characterization of the renal IGRS [10,12], suggest that this receptor could be the target of imidazoline derivatives for regulation of sodium influx. This hypothesis is supported by the low drug concentrations required for the detection of the functional effect and by the order of potency for inhibition of ^{22}Na influx in isolated proximal cells. This order of potency is similar to that observed for inhibition of [^{3}H]idazoxan binding to purified basolateral membranes from rabbit proximal tubule [10–12]. However, at present, the difficulty to define specific antagonists at IGRS for reversing the imidazoline effect does not permit to confirm this hypothesis.

When this work was in progress, we found that amiloride [12] and derivatives such as EIPA and phenamylamiloride [29], displayed a high affinity (in the nanomolar range) for renal IGRS. Similar results have been also reported for nonadrenergic [^{3}H]idazoxan binding sites in rabbit adipocytes [24] and pig kidney [25]. This direct interaction of amiloride and its derivatives with both IGRS and Na^+/H^+ antiporter makes difficult the characterization of the Na^+ transport system involved in imidazolines effect by using amiloride as a tool. Consequently, in the attempt to define whether imidazoline derivatives inhibit Na^+/H^+ antiporter, we performed two sets of supplementary experiments. In the first one, we investigated the effect of idazoxan on $^{22}Na^+$ influx after acidification of the extracellular pH. In these experimental conditions, where the activity of the Na^+/H^+ antiporter is abolished [19], idazoxan did not modify the ^{22}Na entry. In the second series of experiments, we tested the effect of idazoxan and cirazoline on the regulation of the intracellular pH in isolated proximal tubule cells. In these cells, where the Na^+-induced intracellular alkalinization reflects, almost completely, the activity of the Na^+/H^+ antiporter [19], we found that cirazoline and idazoxan inhibited the velocity of H^+ efflux after addition of Na^+ to the medium. Moreover, the order of magnitude for inhibition of cell realkalinization was similar that observed for the decrease in $^{22}Na^+$ influx. These data, along with the concomitant inhibition of $^{22}Na^+$ entry and cell realkalinization, support the hypothesis that imidazoline derivatives decrease the Na^+ influx in the proximal cells through the inhibition of the Na^+/H^+ antiporter. However, the magnitude of the changes in $^{22}Na^+$ influx and intracellular pH, suggest that the modulation of the Na^+/H^+ antiporter activity could be secondary to another major biological effect induced by imidazoline derivatives. In conclusion, we demonstrated that imidazoline derivatives modulate Na^+ influx independently of the interaction with alpha-2 adrenergic receptors. These findings supply new insights for the explanation of discordant results on the role of alpha-2 adrenergic receptors on sodium transport in the renal proximal tubule. Moreover, they could be the starting point for the characterization of the functional activity and the second messenger system controlled by the renal IGRS.

References

1 Bello-Reuss, E., Trevino, D.L. and Gottschalk, C.G. (1976) J. Clin. Invest. 57, 1104–1107.

2 Prosnitz, E.H. and DiBona, G.F. (1978) Am. J. Physiol. 235, F557–F563.

3 Hesse, I.F.A. and Johns, E.J. (1984) J. Physiol. 352, 527–538.

4 Osborn, J.L., Holdass, H., Thames, M.D. and DiBona, G.F. (1983) Circ. Res. 53, 298–305.

5 Pettinger, W.A., Smyth, D.D. and Umemura, S. (1985) J. Cardiovasc. Pharmacol. 7 (Suppl. 8), 524–527.
6 Smyth, D.D., Umemura, S. and Pettinger, W.A. (1984) Am. J. Physiol. 247, F680–F685.
7 Bylung, D. (1988) Trends Pharmacol. Sci. 91, 356–361.
8 Hesse, F.A. and Johns, E.J. (1985) J. Pharmacol. 84, 715–724.
9 Insel, P.A., Snavely, M.D., Healy, D.P., Münzel, P.A., Potenza, C.L. and Nord, E.P. (1985) J. Cardiovasc. Pharmacol. 7, S9–S17.
10 Coupry, I., Podevin, R.A., Dausse, J.P. and Parini, A. (1987) Biochem. Biophys. Res. Commun. 147, 1055–1060.
11 Coupry, I., Lachaud, V., Podevin, R.A., Koenig, E. and Parini, A. (1989) Am. J. Hypertension 2, 468–470.
12 Parini, A., Coupry, I., Graham, R.M., Uzielli, I., Atlas, D. and Lanier, S.M. (1989) J. Biol. Chem. 264, 11874–11878.
13 Atlas, D. and Burstein, Y. (1984) Eur. J. Biochem. 144, 287–293.
14 Diamant, S. and Atlas, D. (1986) Biochem. Biophys. Res. Commun. 134, 184–190.
15 Felsen, D., Ernsberger, P.R., Meeley, M.P. and Reis, D.J. (1987) Eur. J. Pharmacol. 142, 453–455.
16 Bousquet, P., Feldman, J. and Atlas, D. (1986) Eur. J. Pharmacol. 124, 167–170.
17 Poujeol, P. and Vandewalle, A. (1985) Am. J. Physiol. 249, F461–F471.
18 Bradford, M. (1976) Anal. Biochem. 72, 248–254.
19 Bidet, M., Tauc, M., Merot, J., Vandewalle, A. and Poujeol, P. (1987) Am. J. Physiol. 253, F935–F944.
20 Roos, A. and Boron, W.F. (1981) Physiol. Rev. 61, 296–434.
21 Paradiso, A.M., Tsien, R.Y. and Machen, T.E. (1984) Proc. Natl. Acad. Sci. USA 81, 7436–7440.
22 Bidet, M., Merot, J., Tauc, M. and Poujeol, P. (1987) Am. J. Physiol. 253, F945–F951.
23 Strandhoy, J.W. and Gesek, A. (1989) Kidney Int. 35, 488.
24 Langin, D. and Lafontan, M. (1989) Eur. J. Pharmacol. 159, 199–203.
25 Vigne, P., Lazdunski, M. and Frelin, C. (1989) Eur. J. Pharmacol. 160, 295–298.
26 Roman, R.J., Cowley, A.W. and Lechene, C. (1979) J. Pharmacol. Exp. Ther. 211, 385–393.
27 Nord, E.P., Howard, M.J., Hafezi, A., Moradeshagi, P., Vaystub, S. and Insel, P.A. (1987) J. Clin. Invest. 80, 1755–1762.
28 Ganapathy, M.E., Leibach, F.H., Mahesh, V.B., Devoe, L.D. and Ganapathy, V. (1986) Biochem. Pharmacol. 35, 3989–3994.
29 Coupry, I., Atlas, D., Podevin, R.A., Uzielli, I. and Parini, A. (1990) J. Pharmacol. Exp. Ther. 252, 293–299.