Non-selective Effects of Amiloride and Its Analogues on Ion Transport Systems and Their Cytotoxicities in Cardiac Myocytes

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ABSTRACT—The effects of amiloride and its analogues (3',4'-dichlorobenzamil (DCB), 2',4'-dimethylbenzamil (DMB), 5-(N-ethyl-N-isopropyl)amiloride (EIPA) and 5-(N-methyl-N-isobutyl)amiloride (MIBA)) on cardiac ion transporters (Na⁺/Ca²⁺ exchanger, Na⁺/H⁺ exchanger, Na⁺ pump and Ca²⁺ pump) and their cytotoxicities were tested in cardiac myocytes. All the tested compounds showed concentration-dependent inhibitory effects on the ion transporters studied in canine cardiac sarcolemmal vesicles. The concentrations (μM) of amiloride, DCB, DMB, EIPA and MIBA required to produce 50% inhibition were > 1000, 19, 10, 83 and 84, respectively, for the Na⁺/Ca²⁺ exchanger; 130, 73, 63, 16 and 14 for the Na⁺/H⁺ exchanger; > 1000, 72, > 300, > 300 and > 300 for the Na⁺ pump; and > 1000, 37, 93, 90 and 70 for the Ca²⁺ pump, respectively. Furthermore, these agents induced cell death in isolated rat cardiac myocytes and the 50% lethal concentrations (μM) were > 1000, 9.2, 30, 16 and 17, respectively. These findings demonstrate that amiloride and its analogues have non-selective inhibitory effects on cardiac ion transporters and cytotoxicity in cardiomyocytes. When these drugs are employed as experimental tools to investigate the involvement of ion transporters in cell functions, the results must be interpreted with caution.

Keywords: Amiloride analogue, Ion transporter, Cytotoxicity, Cardiac myocyte

It is widely accepted that most cellular functions are regulated by certain extracellular and intracellular ions. Ca²⁺ is most usually involved, because there are many Ca²⁺-dependent enzymes (protein kinase C, calmodulin kinase, phospholipase C, calpain and so on) that play very important roles in cellular function. Therefore, many Ca²⁺ transport systems are present in the plasma membrane, and there is a fourfold difference between the intracellular and extracellular Ca²⁺ concentration. Voltage-dependent and receptor-coupled Ca²⁺ channels, Ca²⁺ pump and Na⁻/Ca²⁺ exchanger directly alter the intracellular Ca²⁺ concentration, while other transport systems that regulate the intracellular Na⁺ concentration (Na⁺ pump, Na⁺ channel and Na⁺/H⁺ exchanger) indirectly alter the Ca²⁺ concentration via the Na⁺/Ca²⁺ exchanger. Therefore, many ion transporters affect the intracellular Ca²⁺ concentration (1). This implies that changes in the activities of cellular ionic regulation systems are a very important factor in estimating cell function in biochemical and pharmacological studies. In such studies, the effect of the cellular ionic regulation system on cellular function has been studied by using drugs that definitely inhibit the transporter in question without consideration of the specificities of these inhibitors. This may lead to misunderstanding of the particular cellular function because there is a possibility that these drugs might also inhibit ion transporters other than the target system.

Amiloride and its analogues are employed as tools in the study of ion transport systems (2, 3). Amiloride was initially demonstrated to be a diuretic because of its inhibitory effect on the Na⁺ channel present in urinary epithelia (4), but subsequent studies have shown that it is a multipotent drug that inhibits a variety of other ion transporters and enzymes (2, 3). Various amiloride analogues have subsequently been synthesized and some "more specific" inhibitors for ion transporters, for example, 3',4'-dichlorobenzamil (DCB) and 2',4'-dimethylbenzamil (DMB) for the Na⁺/Ca²⁺ exchanger (5), 5-(N-ethyl-N-isopropyl)amiloride (EIPA) and 5-(N-methyl-N-isobutyl)amiloride (MIBA) for the Na⁺/H⁺ exchanger (6), have been discovered and used in many experiments. In this study, we tested the ability of these compounds to interfere with other ion transporters in order to examine their specificities. Although similar studies have already been published (3),
it was not clear from these whether the selectivities of the inhibitory effects of these compounds for ion transporters reflected differences in their effects on the ion transporters themselves or in the characteristics of the organs studied, because the samples for assay were taken from various organs. Therefore, we examined the inhibitory effects of amiloride and four of its analogues (DCB, DMB, EIPA, MIBA) on the Na\(^+\)/Ca\(^{2+}\) exchanger, Na\(^+\)/H\(^+\) exchanger, Na\(^+\) pump and Ca\(^{2+}\) pump using only one kind of membrane prepared from canine heart. Moreover, the ability of amiloride and its analogues to injure isolated rat cardiac myocytes was tested in order to examine the direct effects of these agents on the cell preparation.

MATERIALS AND METHODS

Materials

Amiloride was purchased from Sigma (St. Louis, MO, USA), and amiloride analogues were synthesized in our synthetic chemistry department as described previously (7). Figure 1 shows the chemical structures of amiloride and the analogues used in this study. \(^{22}\)NaCl and \(^{45}\)CaCl\(_2\) were purchased from Amersham (Arlington Heights, IL, USA). All other reagents were obtained from commercial sources and were of the highest purity available.

Isolation of canine cardiac membrane fractions and rat cardiac myocytes

The cardiac sarcolemmal vesicles (SLV) and sarcoplasmic reticular vesicles (SRV) were isolated from canine heart by discontinuous sucrose density gradient centrifugation of a crude membrane preparation according to the procedures of Jones (8) and Chamberlain and Fleischer (9), respectively, and they were stored at -80°C until use.

Isolated cardiac myocytes were obtained from rats according to a previously described procedure with some modifications (10). Hearts were excised from anesthetized rats and perfused via the aorta with Ca\(^{2+}\)-free HEPES-Tyrode solution supplemented with 0.05% collagenase for 30–40 min at 37°C. The cell suspension was stored at room temperature until use. Only Ca-tolerant and rod-shaped myocytes were used for studies of the drug cytotoxicities.

Measurement of membrane ion transporter activities

In this study, membrane ion transporter activities are presented as activities per mg protein. These were determined by the procedure described by Lowry et al. (11). Na\(^+\)/Ca\(^{2+}\) exchange activity was assayed as intracellular Na (Na\(^+\))-dependent \(^{45}\)Ca uptake by a previously described procedure with minor modifications (12). Briefly, SLV were preincubated in a solution containing 160 mM NaCl and 20 mM MOPS-Tris (pH 7.4) and then loaded with Na\(^+\) for 30 min at 37°C. Total \(^{45}\)Ca uptake was initiated by incubation of this suspension (5 μl) in an assay mixture (250 μl) containing 160 mM KCl, 20 μM \(^{45}\)CaCl\(_2\) (0.3 μCi), 0.4 μM valinomycin, 20 mM MOPS-Tris (pH 7.4) for 2 sec at 37°C with or without the test drug. \(^{45}\)Ca uptake was halted by the addition of a cold solution containing 160 mM KCl and 1 mM LaCl\(_3\). This solution was rapidly filtered under vacuum using Whatman GF/A glass fiber filters, followed by rinsing again with a solution containing 160 mM KCl and 1 mM LaCl\(_3\). The radioactivity trapped on the filters was determined by liquid scintillation spectroscopy. Na\(^+\)-independent \(^{45}\)Ca uptake was measured by substitution of KCl for NaCl in the assay mixture and wash solution, and net Na\(^+\)-dependent \(^{45}\)Ca uptake was calculated as the difference between the

![Fig. 1. The chemical structures of amiloride and its analogues. DCB: 3',4'-dichlorobenzamil, DMB: 2',4'-dimethylbenzamil, EIPA: 5-(N-ethyl-N-isopropyl)amiloride, MIBA: 5-(N-methyl-N-isobutyl)amiloride.](image-url)
total uptake and Na\(^+\)-independent uptake.

Na\(^+\)/H\(^+\) exchanger activity was assayed by measuring \(^{22}\)Na uptake driven by a transmembrane pH gradient according to a previously described procedure with minor modifications (13). In brief, SLV were preincubated in a solution containing 100 mM mannitol, 40 mM 2-(N-morpholino)ethylsulfonic acid, 20 mM α-methylglucamine and 1 mM EGTA (pH 5.9). This was loaded with H\(^+\) and left overnight at 4°C. Total \(^{22}\)Na uptake was initiated by incubation of this suspension (5 μl) in an assay mixture (200 μl) containing 100 mM mannitol, 40 mM 2-(N-morpholino)ethylsulfonic acid, 20 mM α-methylglucamine, \(^{22}\)Na gluconate (1 μCi) and 1 mM EGTA (pH 7.4) for 20 sec at 25°C with or without the test drug. \(^{22}\)Na uptake was halted by the addition of a cold solution containing 100 mM mannitol, 100 mM MgCl\(_2\), 8 mM HEPES and 4 mM Tris (pH 7.4). This solution was rapidly filtered under vacuum using nitrocellulose filters, followed by rinsing twice with the same solution. The radioactivity trapped on the filters was determined by liquid scintillation spectroscopy. H\(^+\)-independent \(^{22}\)Na uptake was measured by replacing the reaction solution with one containing 100 mM mannitol, 37.5 mM HEPES, 22.5 mM α-methylglucamine, \(^{22}\)Na gluconate (1 μCi) and 1 mM EGTA (pH 7.4). Net H\(^+\)-dependent \(^{22}\)Na uptake was calculated as the difference between the total uptake and H\(^+\)-independent uptake.

Na\(^+\) pump and Ca\(^{2+}\) pump activities were measured by a spectrophotometric assay (14). For the assay of Na\(^+\) pump activity, canine SLV were added to the reaction mixture (50 mM Tris-HCl, 100 mM NaCl, 20 mM KCl, 3 mM MgCl\(_2\), 3 mM ATP, with or without 0.2 mM ouabain and with or without the test drug, pH 7.4) and incubated at 37°C. After 20 min, the reaction was stopped by the addition of 10% trichloroacetic acid (TCA), and the amount of inorganic phosphate released was determined. The specific activity of the Na\(^+\) pump was calculated as the difference between the activities measured in the presence and in the absence of ouabain.

For the assay of Ca\(^{2+}\) pump activity, canine SRV were added to the reaction mixture (40 mM Tris-HCl, 100 mM KCl, 3 mM MgCl\(_2\), 2 mM ATP, 0.2 mM ouabain, plus either 0.04 mM CaCl\(_2\) or 0.5 mM EGTA, pH 7.4) and incubated at 37°C. After 20 min, the reaction was stopped by the addition of 10% TCA, and the amount of inorganic phosphate released was determined. The specific activity of the Ca\(^{2+}\) pump was calculated as the difference between the activities measured in the presence of CaCl\(_2\) and EGTA.

**Assay for drug cytotoxicities**

Drug cytotoxicities were determined by measuring the rate of drug-induced cell death. A suspension of isolated myocytes was poured into the wells of a 96-well multiplate, and the plate was allowed to stand until the cells adhered to the bottom of the wells. The number of rod-shaped myocytes was counted with a hemocytometer before and after 20 min of drug treatment at room temperature, and the mortality was calculated as follows:

\[
\text{Mortality (\%)} = \frac{\text{Number of rod-shaped cells after 20 min of drug treatment}}{\text{Number of rod-shaped cells before addition of tested drugs}} \times 100
\]

The IC\(_{50}\) values for the ion transporter activities and LC\(_{50}\) values for the intact cardiomyocytes were calculated by linear regression analysis.

**RESULTS**

*Inhibition of the Na\(^+\)/Ca\(^{2+}\) exchanger*

Figure 2 shows the concentration-response curves for the effects of amiloride and its analogues on the Na\(^+\)/Ca\(^{2+}\) exchanger in cardiac sarcolemmal vesicles. In the forward mode, the Na\(^+\)/Ca\(^{2+}\) exchanger mediates Ca efflux that is coupled to an inwardly directed Na\(^+\) gradient, while in reverse mode, it mediates Ca influx coupled to an outwardly directed Na\(^+\) gradient. In this study, we tested the activity of the Na\(^+\)/Ca\(^{2+}\) exchanger in the reverse mode. All four analogues, but not amiloride, showed concentration-dependent inhibitory effects. Amiloride at the highest concentration of 1 mM only produced 31.6±1.0% inhibition. Table 1 shows the IC\(_{50}\) values for the four analogues calculated by linear regression analysis.

Fig. 2. Concentration-response curves for the inhibitory effects of amiloride and its analogues on the canine cardiac sarcolemmal Na\(^+\)/Ca\(^{2+}\) exchanger. Each point represents the mean±S.E.M. of 3–5 observations. The basal activity of the Na\(^+\)/Ca\(^{2+}\) exchanger was 2.7±1.3 nmol Ca/mg protein/sec. Amiloride (○), DCB (●), DMB (□), EIPA (■), MIBA (▲).
The IC50 values of DMB, DCB, EIPA and MIBA were 10 pM, 19 pM, 83 pM and 84 pM, respectively. DMB was therefore the most potent inhibitor among the tested compounds.

**Table 1.** Inhibitory activities of amiloride and its analogues for several ion transporters in canine heart membrane preparations and their cytotoxicities in isolated rat cardiac myocytes

| Drugs  | IC50 (μM) for Na+/Ca2+ exchanger | IC50 (μM) for Na+/H+ exchanger | IC50 (μM) for Na+ pump | LC50 (μM) for cardiac myocytes |
|--------|--------------------------------|--------------------------------|------------------------|-------------------------------|
| Amiloride | >1000 (66–220) | 130 (53–150) | >1000 (53–110) | >1000 (30–46) |
| DCB | 19 (12–26) | 73 (48–90) | 72 (62–140) | 9.2 (7.0–12) |
| DMB | 10 (7.7–13) | 63 (8.3–25) | >300 (48–160) | 30 (21–43) |
| EIPA | 83 (61–110) | 16 (9.7–20) | >300 (50–100) | 17 (10–41) |
| MIBA | 84 (75–94) | 14 (9.7–20) | >300 (50–100) | 17 (10–41) |

IC50 and LC50 values were calculated by linear regression analysis. The numbers in parentheses represent 95% confidence limits.

The IC50 values of DMB, DCB, EIPA and MIBA were 10 μM, 19 μM, 83 μM and 84 μM, respectively. DMB was therefore the most potent inhibitor among the tested compounds.

**Inhibition of the Na+/H+ exchanger**

Figure 3 shows the effects of amiloride and its analogues on the Na+/H+ exchanger in cardiac sarcolemmal vesicles. All compounds possessed concentration-dependent inhibitory effects on the Na+/H+ exchanger. MIBA was the most potent inhibitor with an IC50 value of 14 μM (Table 1). The IC50 value of amiloride was 130 μM. MIBA was about 9 times more potent than amiloride, EIPA about 8 times, DMB about 2 times and DCB about 1.8 times as potent. The order of inhibitory potency of these compounds for the Na+/H+ exchanger was different from that for the Na+/Ca2+ exchanger.

**Inhibition of the Na+ pump**

Figure 4 shows the effects of amiloride and its analogues on the Na+ pump in cardiac sarcolemmal vesicles. DCB and DMB possessed inhibitory effects on the Na+ pump, but EIPA, MIBA and amiloride actually did not. The IC50 values of DCB was 72 μM (Table 1). The IC50 values were calculated by linear regression analysis.
value of DMB could not be calculated, because the expected IC50 value exceeded its solubility.

Inhibition of the Ca2+ pump

We also tested the effects of amiloride and its analogues on the Ca2+ pump in sarcoplasmic reticular vesicles (Fig. 5). Although the effects were not as well defined as for the Na+ pump, all the analogues tested inhibited the Ca2+ pump in a concentration-dependent manner, and amiloride at the highest concentration of 1 mM showed 31% inhibition. Among the analogues, DCB inhibited the Ca2+ pump most potently with an IC50 value of 37 μM (Table 1). The potencies of DMB, EIPA and MIBA were similar. Although the primary structure of the Na+ pump protein has been reported to be similar to that of the Ca2+ pump and to have >30% homology (15), the order of potency for amiloride and its analogues in inhibiting the Ca2+ pump was different from that for the Na+ pump.

Cytotoxicity in isolated cardiac myocytes

Finally, to determine what effect amiloride and its analogues have on cardiac myocytes, we examined their cytotoxicities in isolated cardiac myocytes and determined the cell mortality rates induced by these drugs. As shown in Fig. 6, all of the analogues examined, except amiloride, possessed concentration-dependent cytotoxic effects in cardiac myocytes in the following order: DCB > EIPA = MIBA > DMB. The LC50 values were 9.2 μM for DCB, 16 μM for EIPA, 17 μM for MIBA and 30 μM for DMB (Table 1). In addition, we examined whether the Na+ pump inhibitor ouabain and the Ca2+ pump inhibitor cyclopiazonic acid possessed cytotoxic effects on cardiomyocytes. Neither ouabain nor cyclopiazonic acid caused cell death at concentrations up to 10 μM (data not shown).

DISCUSSION

We demonstrated that amiloride and its analogues had inhibitory effects on several cardiac ion transporters and that their relative selectivities for the inhibitory effects were very different.

Amiloride only had an effect on the Na+/H+ exchanger, and its IC50 value was 130 μM. This value was very similar to that observed in an earlier study (13). Kleymann and Cragoe reported that an amiloride-sensitive Na+ channel was present in the epithelium, that its IC50 value for amiloride was 0.35 μM and that amiloride produced diuresis due to this effect (3). Furthermore, they reported that amiloride inhibited the voltage-dependent Na+ channel and the L-type voltage-dependent Ca2+ channel with IC50 values of 600 μM and 90 μM, respectively. Therefore, if amiloride is used in an experiment at a concentration of 100 to 1000 μM, inhibition of the Na+/H+ exchanger, voltage-dependent Na+ channel and Ca2+ channel can all be expected. Moreover, this agent has been observed to exert a depressant action on cardiac Na+ channels at 1000 μM in guinea pig ventricular muscle (16). These findings suggest that we can employ amiloride as a selective epithelial Na+ channel inhibitor at a concen-
DCB and DMB have been recognized as inhibitors of the Na\(^+\)/Ca\(^{2+}\) exchanger (5), and we have confirmed that these two compounds have inhibitory effects with similar potencies on the Na\(^+\)/Ca\(^{2+}\) exchanger in cardiac sarcolemma. Furthermore, we observed that these compounds also exert inhibitory effects on other ion transporters with similar potencies and that, above all, DCB inhibited all four transporters that we studied. This implies that the involvement of other ion transporters cannot be excluded when DCB is used as a Na\(^+\)/Ca\(^{2+}\) exchanger inhibitor. Since no more selective inhibitors for the Na\(^+\)/Ca\(^{2+}\) exchanger than DCB have as yet been developed to prove the participation of the Na\(^+\)/Ca\(^{2+}\) exchanger in certain physiological functions, inhibitors specific for the other ion transporters should be used in conjunction with DCB.

EIPA and MIBA have been employed for the inhibition of the Na\(^+\)/H\(^-\) exchanger in pharmacological studies. The changes in intracellular pH caused by growth factors are antagonized by EIPA and MIBA (17). This means that the Na\(^+\)/H\(^-\) exchanger is involved in the regulation of intracellular pH and that EIPA and MIBA are useful tools for studies of intracellular pH regulation in cell preparations. However, we have shown that EIPA and MIBA also inhibit the Na\(^+\)/Ca\(^{2+}\) exchanger and the Ca\(^{2+}\) pump with the same potency (IC\(_{50}\) values: 70–90 \(\mu\)M) as that for the Na\(^+\)/H\(^-\) exchanger in membrane preparations. EIPA has been reported to inhibit the voltage-dependent Na\(^+\) channel of rat brain synaptosomes with an IC\(_{50}\) value of 6.1 \(\mu\)M (18). These findings suggest that EIPA and MIBA are not specific inhibitors of the Na\(^+\)/H\(^-\) exchanger; therefore, even if Na influx into cells is inhibited by EIPA or MIBA, we can not conclude that this is solely due to the involvement of the Na\(^+\)/H\(^-\) exchanger, at least in membrane preparations.

In addition to the ion transporters that we studied, there are several other important ones such as the voltage-dependent Ca\(^{2+}\) channel. Garcia et al. reported that amiloride, DCB, EIPA and MIBA inhibit \([H]diltiazem\)-binding in cardiac membranes and depolarization-induced Ca uptake in GH3 rat anterior pituitary cells (19). Pierce et al. also reported that these four agents depress cardiac contractility in the rat right ventricular wall and that these effects may involve a complex inhibition of Ca influx and K efflux in addition to a nonselective cation current (20). These findings support our proposal that amiloride and its analogues are non-selective inhibitors.

We examined the shape change from rod-shaped to round to determine the cytotoxicity of amiloride and its analogues in isolated rat cardiac myocytes. It has been suggested that this shape change indicates a loss of viability (21). We showed that amiloride analogues possessed cytotoxic effects on the myocardial cells and that DCB was the most cytotoxic of the four analogues. The reason for this is not clear. Since the Na\(^+\) pump inhibitor ouabain and the Ca\(^{2+}\) pump inhibitor cyclopiazonic acid do not injure cardiac myocytes (data not shown), inhibition of the Na\(^+\) or Ca\(^{2+}\) pumps does not seem to be the mechanism of action. These drugs may not exert their cytotoxicities by an independent inhibitory effect on one ion transporter but by a combination of several additive or synergistic effects. They may also have effects other than inhibitory ones on the ion transporters. One possibility is that these analogues may have a direct effect on oxidative phosphorylation (22) and protein synthesis (6), thereby inducing death in cardiac myocytes. At present, the nature of the cytotoxicity produced by amiloride analogues is not clear.

Amiloride and its analogues are indispensable drugs for studies of cardiac physiology, because most heart functions are dependent on intracellular ions. These drugs have inhibitory effects on ion transporters and affect intracellular ion concentrations in cardiac myocytes. Many investigators have used amiloride, EIPA, MIBA or 5-(N,N-hexamethylene)amiloride (HMA), and DCB or DMB to examine the physiological role of the Na\(^+\)/H\(^+\) and Na\(^+\)/Ca\(^{2+}\) exchangers in cardiac injury produced by ischemia-reperfusion or hypoxia-reoxygenation (23–27). In interpreting the results of such studies, satisfactory conclusions might not always be reached because of plural logic originating from the non-selective inhibitory effects of these drugs on ion transporters. Therefore, specific inhibitors for ion transporters, unlike amiloride or its analogues, are necessary to obtain conclusive evidence and will need to be developed.

In conclusion, amiloride and its analogues are non-selective inhibitors of cardiac ion transporters. They also exert cytotoxic effects on cardiac myocytes with a potency similar to that for the inhibitory effects on ion transporters. Therefore, when these drugs are employed as experimental tools to examine the involvement of ion transporters in cell functions, interpretation of the experimental results may be very difficult and must be evaluated carefully.

REFERENCES
1 McCormack JG, Boyett MR, Jewell BR and Orchard CH: Ion movement and contractility in heart cells. Trends Pharmacol Sci 9, 333–345 (1988)
2 Kleymann TR and Cragoe EJ Jr: Amiloride and its analogs as tools in the study of ion transport. J Membr Biol 105, 1–21 (1988)
3 Kleymann TR and Cragoe EJ Jr: Cation transport probes: The amiloride series. Methods Enzymol 191, 739–755 (1990)
4 Sariban-Sohraby S and Benos DJ: The amiloride-sensitive
sodium channel. Am J Physiol 250, C175–C190 (1986)
5 Kaczorowski GJ, Barros F, Dethmers JK and Trumble MJ: Inhibition of Na⁺/Ca²⁺ exchange in pituitary plasma membrane vesicles by analogues of amiloride. Biochemistry 24, 1394–1403 (1985)
6 Zhung Y, Cragoe EJ Jr, Shaikewitz T, Glaser L and Cassel D: Characterization of potent Na⁺/H⁺ exchange inhibitors from the amiloride series in A431 cells. Biochemistry 23, 4481–4488 (1984)
7 Cragoe EJ Jr, Woltersdorf OW Jr, Bicking JB, Kwong SF and Jones JH: Pyrazine diuretics. II. N-Amidino-3-amino-5-substituted 6-halopyrazinecarboxamides. J Med Chem 10, 66–75 (1967)
8 Jones LR: Rapid preparation of canine cardiac sarcosomal vesicles by sucrose flotation. Methods Enzymol 157, 85–91 (1988)
9 Chamberlain BK and Fleischer S: Isolation of canine cardiac sarcoplasmic reticulum. Methods Enzymol 157, 91–99 (1988)
10 Donck LV, Pauwels PJ, Vandeplasse G and Borgers M: Isolated rat cardiac myocytes as an experimental model to study calcium overload: The effect of calcium-entry blockers. Life Sci 38, 765–772 (1986)
11 Lowry OH, Rosebrough NJ, Farr AL and Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 193, 265–275 (1951)
12 Reeves JP and Sutko JL: Competitive interactions of sodium and calcium with the sodium-calcium exchange system of cardiac sarcosomal vesicles. J Biol Chem 258, 3178–3182 (1983)
13 Seiler SM, Cragoe EJ Jr and Jones LR: Demonstration of a Na⁺/H⁺ exchange activity in purified canine cardiac sarcosomal vesicles. J Biol Chem 260, 4869–4876 (1985)
14 Fiske CH and Subbarow Y: The colorimetric determination of phosphorus. J Biol Chem 66, 375–400 (1925)
15 Shull GE, Schwartz A and Lingrel JB: Amino-acid sequence of the catalytic subunit of the (Na⁺+K⁺)ATPase deduced from a complementary DNA. Nature 316, 691–695 (1985)
16 Lai Z-T, Hotokebuchi N, Cragoe EJ Jr and Nishi K: Effects of S-(N,N-hexamethylene)amiloride on action potentials, intracellular Na, and pH of guinea pig ventricular muscle in vitro. J Cardiovasc Pharmacol 23, 259–267 (1994)
17 Gaidano G, Ghigo D, Schena M, Bergui L, Treves S, Turrini F, Cappio FC and Bosia A: Na⁺/H⁺ exchange activation mediates the lipopolysaccharide-induced proliferation of human B lymphocytes and is impaired in malignant B-chronic lymphocytic leukemia lymphocytes. J Immunol 142, 913–918 (1989)
18 Velly J, Grima M, Decker N, Cragoe EJ Jr and Schwartz J: Effects of amiloride and its analogues on [³H]batrichotoxinin-A 20-α benzoate binding, [³H]tetraclaine binding and Na⁺ influx. Eur J Pharmacol 149, 97–105 (1988)
19 Garcia ML, King VF, Shevell JL, Slaughter RS, Suarez-Kurta G, Winquist RJ and Kaczorowski GI: Amiloride analogs inhibit L-type calcium channels and display calcium entry blocker activity. J Biol Chem 265, 3763–3771 (1990)
20 Pierce GN, Cole WC, Liu K, Massaelli H, Maddaford TG, Chen YJ, McPherson CD, Jain S and Sonntag D: Modulation of cardiac performance by amiloride and several selected derivatives of amiloride. J Pharmacol Exp Ther 265, 1280–1291 (1993)
21 Borgers M, Donck LV and Vandeplasse G: Pathophysiology of Cardiomyocytes. In Calcium Antagonists: Pharmacology and Clinical Research, Edited by Vanhoutte PM, Paoletti R and Govoni S, Vol 522, pp 433–453, The New York Academy of Sciences, New York (1988)
22 Soltoff SP, Cragoe EJ Jr and Mandel LJ: Amiloride analogues inhibit proximal tubule metabolism. Am J Physiol 250, C744–C749 (1986)
23 Karmazyn M, Ray M and Haist JV: Comparative effects of Na⁺/H⁺ exchange inhibitors against cardiac injury produced by ischemia/reperfusion, hypoxia/reoxygenation, and the calcium paradox. J Cardiovasc Pharmacol 21, 172–178 (1993)
24 Moffat MP and Karmazyn M: Protective effects of the potent Na⁺/H⁺ exchange inhibitor methylisobutyl amiloride against post-ischemic contractile dysfunction in rat and guinea-pig hearts. J Mol Cell Cardiol 25, 959–971 (1993)
25 Scholz W, Albus U, Linz W, Martorana P, Lang HJ and Scholzens BA: Effects of Na⁺/H⁺ exchange inhibitors in cardiac ischemia. J Mol Cell Cardiol 24, 731–740 (1992)
26 Tanj M: Mechanisms of Ca²⁺ overload in reperfused ischemic myocardium. Annu Rev Physiol 52, 543–559 (1990)
27 Brown L, Cragoe EJ Jr, Abel KC, Manley SW and Bourke JR: Amiloride analogues induced responses in isolated rat cardiovascular tissues by inhibition of Na⁺/Ca²⁺ exchange. Naunyn Schmiedebergs Arch Pharmacol 344, 220–224 (1991)