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Short Communication

**CAPSAICIN-INDUCED ACTIVATION OF ERYTHROCYTE
MEMBRANE SODIUM/POTASSIUM AND CALCIUM ADENOSINE
TRIPHOSPHATASES**

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Abstract: Capsaicin is the pungent ingredient present in hot peppers of the genus *Capsicum*. Capsaicin's effect on sensory neurons has been well studied; however, its effect on non-neuronal cells is still not fully understood. This study was undertaken to evaluate the effect of capsaicin on erythrocyte membrane enzymes: Na⁺/K⁺-ATPase and Ca²⁺-ATPase. Treatment with capsaicin (0.01-100μM) caused a transient increase in the activities of both enzymes; the effect declined at lower concentrations of capsaicin, and no significant effect was observed at 0.01μM capsaicin. The effect of capsaicin was fast with a significant (p<0.01) activation of enzyme activity observed within minutes of incubation. The findings on the effect of capsaicin on human erythrocyte membrane enzymes Na⁺/K⁺-ATPase and Ca²⁺-ATPase signify the importance of the non-neuronal effects of capsaicin, and the need for evaluating the physiological impact of high capsaicin (capsicum) consumption in some regions of the world.

Key Words: Capsaicin, Erythrocyte, Na⁺/K⁺-ATPase, Ca²⁺-ATPase

INTRODUCTION

Capsaicin (Fig. 1) is the pungent ingredient present in a wide variety of plants belonging to the genus *Capsicum*. Use of capsicum is especially popular in hot climates where its dietary intake may be as high as 2.5 g/day [1].

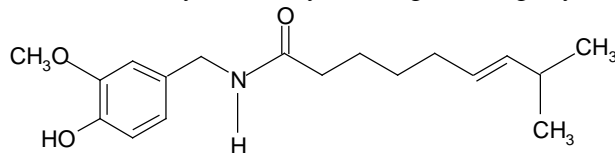


Fig. 1. The structure of capsaicin (*trans*-8-methyl-*N*-vanillyl-6-nonenamide).

Compounds related to capsaicin, collectively referred to as vanilloids, bind specifically to a subclass of sensory neurons called nociceptors, which carry sensation of pain. Prolonged exposure causes nociceptor terminals to become insensitive to capsaicin, as well to other noxious stimuli [2]. Although the effect of capsaicin on neurons has been extensively investigated, few studies have been conducted on its effect on non-neuronal cells. Capsaicin has been reported to inhibit cardiac muscle excitability [3] and visceral smooth muscle excitability [4], induce apoptosis in transformed cells [5] and possess bactericidal properties [6].

In earlier studies, we showed that capsaicin affects human erythrocyte osmotic fragility [7], acetylcholinesterase activity [8] and has anti-oxidative properties [9]. This study was undertaken to evaluate the effect of capsaicin on erythrocyte membrane bound enzymes: Na^+/K^+ -ATPase and Ca^{2+} -ATPase, in view of the importance of these enzymes in human physiological processes.

MATERIALS AND METHODS

Blood collection and isolation of erythrocyte membranes

Venous blood was collected from normal volunteers (n=15) in acid/citrate/dextrose. None of the human volunteers were affected by arthritis, hypertension or diabetes mellitus. The blood sample was centrifuged at 4°C for 10 min at 100 g to remove plasma and buffy coat and the isolated erythrocytes were washed 4-5 times with 0.154 M NaCl. The erythrocyte membranes were isolated following the method of Marchesi and Palade [10].

Determination of Na^+/K^+ -ATPase activity

Na/K -ATPase activity was assayed as described earlier [11]. The final assay mixture contained 0.4 to 0.9 mg membrane protein per ml, 140 mM NaCl, 20 mM KCl, 3 mM MgCl_2 , 30 mM imidazole (pH 7.25), $\pm 5 \times 10^{-4}$ M ouabain and 6 mM ATP. Incubation was carried out for 30 min at 37°C; the reaction was stopped by adding 3.5 ml of a solution containing 0.5 M H_2SO_4 , 0.5% ammonium molybdate and 2% SDS. The amount of liberated inorganic phosphate was estimated by the method of Fiske and Subbarow [12].

Determination of Ca^{2+} -ATPase activity

Ca^{2+} -ATPase activity was assayed as described [13]. 2.25 ml of the assay mixture contained 80 mM NaCl, 15 mM KCl, 3 mM MgCl_2 , 18 mM Tris-HCl (pH 7.4), 0.1 mM ouabain, 0.1 mM EGTA, 0.2 ml of the membrane containing 0.4 to 1.5 mg protein per ml and ± 0.2 mM CaCl_2 . The reaction was initiated by the addition of 0.1 ml of 30 mM ATP. After 30 min at 37°C, the reaction was stopped by adding 3.5 ml of a solution containing 0.5 M H_2SO_4 , 0.5% ammonium molybdate and 2% SDS. The amount of liberated inorganic phosphate was estimated by the method of Fiske and Subbarow [12].

***In vitro* experiments with capsaicin and capsazepine**

The effect of capsaicin on erythrocyte membrane Na^+/K^+ -ATPase and Ca^{2+} -ATPase was studied following the procedure used in our earlier studies [8, 9]. A stock solution (10 mM) of capsaicin (Sigma Chemical Company, USA; 98% pure) was made in 5% ethanol; further dilutions (1mM-0.1 μM) were done with water. The concentration of ethanol was always <0.005% in the final solution. The *in vitro* effect of capsaicin was evaluated by incubating the assay medium in the presence of indicated (10-0.01 μM) final concentration of capsaicin for 60 min at 37°C. This concentration range of capsaicin is in accordance with the results of previous studies on the biological effects (neuronal and non-neuronal) of capsaicin [15-18]. Time-dependent studies were also performed by varying the incubation time from 0-120 min. In parallel control experiments, the assay medium was incubated with solvent alone. Studies were also performed with capsazepine, a known antagonist of capsaicin.

Protein determination

Erythrocyte membrane protein was estimated following the method of Lowry *et al.* [14].

Statistical analysis

The statistical analysis of the data was performed employing ANOVA followed by Students 't' test, with a probability of <0.01 as the level of significance.

RESULTS

In vitro incubation of the erythrocyte membrane with capsaicin (1-100 μM) resulted in a significant activation of erythrocyte membrane Na^+/K^+ -ATPase activity. The effect of capsaicin was concentration dependent: maximum activation was observed at 1-10 μM , while there was a decrease at lower concentrations, and no significant effect at 0.01 μM capsaicin (Fig. 2 A).

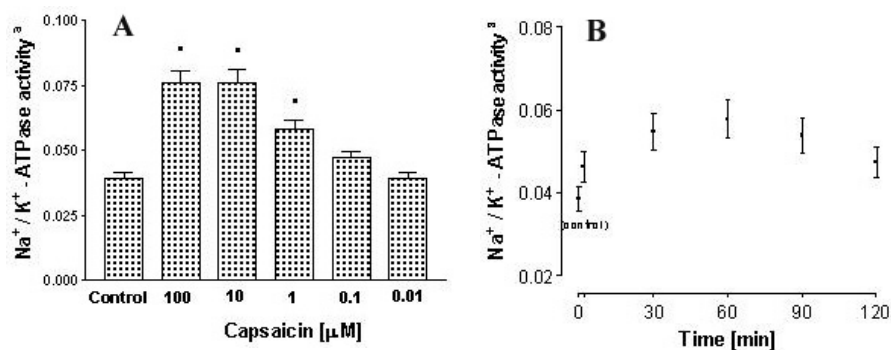


Fig. 2. Effect of capsaicin on erythrocyte Na^+/K^+ -ATPase. A. Concentration dependence. B. time dependence (1 μM capsaicin).^a Na^+/K^+ -ATPase activity in terms of μmoles of Pi liberated/hour/mg of protein at 37°C.

The time dependence of the capsaicin effect on erythrocyte membrane Na^+/K^+ -ATPase is shown in Fig. 2 B. The effect of capsaicin was fast with a significant ($p < 0.01$) activation of enzyme activity observed within minutes of incubation. The maximum effect was observed after 1 hour of incubation; increasing the time beyond 1 hour resulted in a lesser increase in enzyme activity. The Na^+/K^+ -ATPase activity returned to normal level after 2 hours of incubation.

In vitro incubation with capsaicin (1-10 μM) resulted in a significant activation of erythrocyte membrane Ca^{2+} -ATPase activity, an effect that was similar to the effect of capsaicin on Na^+/K^+ -ATPase; the time-dependent effect of capsaicin on Ca^{2+} -ATPase was also similar to that of Na^+/K^+ -ATPase. The results of the assays of the effect of capsaicin on Ca^{2+} -ATPase, concentration and time dependence, are shown in Fig. 3A and 3B.

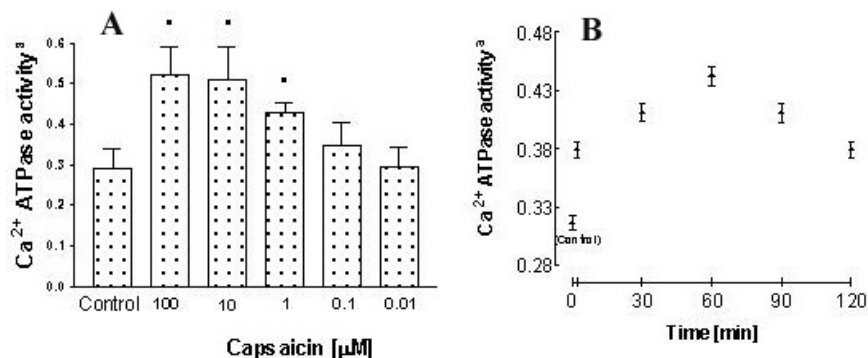


Fig. 3. Effect of capsaicin on erythrocyte Ca^{2+} -ATPase. A. Concentration dependence. B. Time dependence (1 μM capsaicin). ^a Ca^{2+} -ATPase activity in terms of μmoles of Pi liberated/hour/mg of protein at 37°C.

Compared to capsaicin, capsazepine (1 μM) did not affect the activity of Na^+/K^+ - and Ca^{2+} -ATPase activity (data not shown).

DISCUSSION

The neuronal effect of capsaicin is well documented. Nociceptor desensitization by capsaicin has been pharmacologically exploited and has led to the use of capsaicin as a non-steroidal analgesic in chronic painful disorders. The neuronal effects of capsaicin are reported to be mediated through the binding of capsaicin to specific receptors - the vanilloid receptor [2]; non-neuronal cells lack receptors for capsaicin. The neuronal effects of capsaicin are detectable at nanomolar concentrations, while non-neuronal effects require micromolar concentrations. Our findings suggest that the capsaicin effect is non-receptor mediated for two reasons: 1) the effective concentration needed for the effect; 2) the lack of an antagonistic effect of the capsaicin antagonist capsazepine. Evidence is beginning to accumulate on the non-neuronal effects of capsaicin; significantly, these effects are non-specific and extremely heterogeneous [3-9].

The mechanism underlying the non-neuronal effects of capsaicin is unclear; it has been suggested that this lipophilic compound may insert into the cell plasma membrane and alter membrane fluidity [19]. Meddings *et al.* [18] showed that capsaicin alters membrane fluidity in platelets, peritoneal mast cells and red blood cells. It was reported that the fluidising effect of capsaicin on isolated erythrocyte plasma membranes is concentration dependent reaching a saturating point at 200 μM [18]. Capsaicin has been shown to incorporate itself into phospholipid membranes and align with phospholipid acyl chains, perturbing the packing of lipids and affecting thermotropic properties [20]. The capsaicin-induced increase in the activity of the membrane enzymes: Na^+/K^+ -ATPase and Ca^{2+} -ATPase, which we report here, may be explained on the basis of an alteration in the fluidity of the erythrocyte membrane caused by capsaicin. Significantly, two important non-neuronal effects of capsaicin – the inhibition of bacterial growth and platelet aggregation – were also shown to correlate with the membrane effect of capsaicin [21]. The apoptosis inducing effect of capsaicin has also been speculated to be due to the interaction of this compound with the cell membrane [22]. Our findings assume significance since, contrary to the compound's effect on neuronal cells, which is mediated by vanilloid receptors, the non-neuronal effects are non-specific and possibly affect many cell types. To the best of our knowledge, this is the first report on an effect of capsaicin on Na^+/K^+ -ATPase and Ca^{2+} -ATPase on any human non-neuronal cell.

The time-dependence of the capsaicin effect on membrane ATPases is an intriguing observation and difficult to explain. A possible explanation could be that the initial incorporation/interaction of capsaicin with the membrane causes a perturbation of membrane phospholipids, leading to an alteration in the activity of Na^+/K^+ - and Ca^{2+} -ATPase activities; continued exposure to capsaicin permits the membrane to self-organize and regain its native state.

Na^+/K^+ -ATPase and Ca^{2+} -ATPase are integral membrane enzymes, ubiquitously present in all eukaryotic cells, involved in the transport of cations across the plasma membrane. Both these enzymes play an important role in human physiology [23, 24]. The impairment of the sodium pump in the kidney and small intestine can be associated with the pathophysiology of hypertension [25] and chronic diarrhea [26]. Changes in sodium pump activity may also affect excitable tissues such as neurons [27], skeletal muscle cells [28] and the pacemaker fibres of the heart [29]. Our observation of a transient increase in the activity of Na^+/K^+ -ATPase in the presence of capsaicin may have important physiological implications. These findings assume significance in view of the well-known geographical pattern of hot pepper consumption; people living in hot tropical regions of the world prefer 'hot spicy' food compared to those living in temperate climates. The transient increase in the activity of erythrocyte membrane Ca^{2+} -ATPase activity in the presence of capsaicin may also affect human physiology; further work is needed to understand the exact physiological implications.

In conclusion, our findings on the effect of capsaicin on human erythrocyte membrane-bound Na^+/K^+ -ATPase and Ca^{2+} -ATPase signify the importance of the non-neuronal effects of capsaicin, and the need for evaluating the physiological impact of high capsaicin (capsicum) consumption in some regions of the world. The results presented here could also be useful for the understanding of the non-specific effects of capsaicin. Further research is needed to understand the mechanism(s) of the action of capsaicin in its non-selective effects on non-neuronal cells.

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