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Short communication

Bromocriptine stimulates Na^+,K^+-ATPase in renal proximal tubules via the cAMP pathway

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Abstract

The present study was undertaken to examine the effect of dopamine D_2 receptor activation on Na^+,K^+-ATPase activity in rat renal proximal tubule suspension. Bromocriptine, a dopamine D_2 receptor agonist, produced a concentration (10^{-9}–10^{-5} M) dependent stimulation of Na^+,K^+-ATPase activity which was antagonized by pretreating the tubules with domperidone (1 μM), a dopamine D_2 receptor antagonist. Forskolin (1 μM), a direct activator of adenylyl cyclase, inhibited Na^+,K^+-ATPase activity and reversed the stimulation of Na^+,K^+-ATPase activity induced by bromocriptine. Pertussis toxin (200 ng/ml) treatment also abolished the bromocriptine-induced stimulation of Na^+,K^+-ATPase activity. Bromocriptine attenuated forskolin-stimulated cAMP accumulation which was blocked by pertussis toxin treatment of the tubules. The data suggest that dopamine D_2 receptor activation by bromocriptine leads to stimulation of Na^+,K^+-ATPase activity which may be mediated through a pertussis-sensitive G protein and inhibition of adenylyl cyclase in rat renal proximal tubules.

Keywords: Dopamine D_2-like receptor; Na^+ pump; Proximal tubule; Kidney; (Rat)

1. Introduction

Dopamine promotes sodium excretion through hemodynamic changes and via a direct effect on tubular sodium reabsorption. These effects of dopamine result following the activation of cell surface receptors classified as dopamine D_1 and dopamine D_2 receptors (Jose et al., 1992; Lokhandwala and Chen, 1994). The proximal tubular segment of the nephron has been shown to contain both types of dopamine receptors (Felder et al., 1989a; Lokhandwala and Amenta, 1991). Several studies have demonstrated that activation of dopamine D_1 receptors produces inhibition in proximal tubular Na^+,K^+-ATPase located on basolateral membrane and Na^+,H^+ exchanger on brush border membranes (Chen and Lokhandwala, 1993; Felder et al., 1990) and thereby decrease tubular Na^+ absorption. The functional role of dopamine D_2 receptors has been rather controversial. According to some reports, dopamine D_2 receptor activation has no effect on Na^+,K^+-ATPase activity (Chen and Lokhandwala, 1993) whereas others suggest that the dopamine D_2 receptor works in concert with dopamine D_1 receptors and potentiates the inhibitory effect of dopamine D_1 receptor agonists on Na^+,K^+-ATPase activity in the proximal tubules (Bertorello and Aperia, 1990). The dopamine D_1 receptor is linked to adenylyl cyclase and phospholipase C/protein kinase C in a stimulatory manner (Felder et al., 1989a,b; Vyas et al., 1992; Kansra et al., 1995), while the dopamine D_2 receptor is linked to adenylyl cyclase in an inhibitory manner (Gingrich and Caron, 1993). However, the effect of dopamine D_2 receptor agonists on adenylyl cyclase/cAMP in proximal tubular preparations has not been reported. In addition, the phospholipase A_2/arachidonic acid/cytochrome P450 pathway has also been reported to play a role in the signaling system of dopamine D_1 receptors in the kidney (Satoh et al., 1993; Hussain and Lokhandwala, 1996). Because of the controversy over the role of dopamine D_2 receptors in the proximal tubules, we reevaluated the functional status of the dopamine D_2 receptor in terms of the effects of its activation on Na^+,K^+-ATPase activity. We report that the dopamine D_2 receptor agonist, bromocriptine, produced stimulation in Na^+,K^+-ATPase activity in rat proximal tubules. While these studies were underway, a report was published by Yamaguchi et al. (1996) demonstrating the

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stimulatory effect of dopamine D_2 receptor agonists on Na^+,K^+-ATPase activity in murine fibroblast LTK^- cells transfected with a rat dopamine D_{2Long} receptor cDNA.

2. Materials and methods

Male Sprague-Dawley rats of 200–250 g weight (Harlan Sprague-Dawley, Indianapolis, IN, USA) were purchased and housed in plastic cages in an air-conditioned animal care facility. The animals had free access to standard rat chow (Purina Mills, St. Louis, MO, USA) and were given tap water ad libitum.

2.1. Isolation and enrichment of renal proximal tubules

Rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.). Renal cortical tubular suspensions were prepared according to the method of Gesek et al. (1987) with slight modifications adopted in our laboratory (Chen and Lokhandwala, 1993). The renal perfusion was initially performed with modified Krebs-Henseleit buffer (KHB; composition: (mM) NaCl 118, KCl 4, $CaCl_2$ 1.25, $MgCl_2$ 1.2, $NaHCO_3$ 27.2, KH_2PO_4 1, glucose 5 and HEPES 10; pH 7.4). Thereafter, in situ kidney digestion was done by perfusing with 40 ml of KHB-enzyme solution (collagenase type IV, 230 U/ml and hyaluronidase type III, 250 U/ml in KHB). Kidneys were excised, outer cortex was cut, minced and finally incubated with 20 ml of KHB-enzyme solution (collagenase, 460 U/ml and hyaluronidase, 500 U/ml in KHB) under 95% O_2/5% CO_2 until uniformly dispersed. The suspension was filtered through a nylon net (mesh size 105 μm, Spectrum Medical, Los Angeles, CA, USA). The filtrate was centrifuged at $50 \times g$ for 2 min. The pellets were resuspended in ice-cold KHB containing no calcium, 0.12 mM magnesium and 10% calf serum and washed by centrifugation at $250 \times g$ for 5 min and resuspending two times. To the pellet suspension, Ficoll (10 ml of 20%) was added and centrifuged at $250 \times g$ for 15 min. The Ficoll layer was collected and washed with KHB (containing no phosphate and 0.12 mM magnesium) three times. Finally, the pellets were suspended in the same buffer. Protein was assayed using a kit from Pierce (Rockford, IL, USA) and bovine serum albumin was used as standard.

2.2. Na^+,K^+-ATPase assay

Na^+,K^+-ATPase activity was measured by the method of Quigley and Gotterer (1965) and adopted in our laboratory with slight modifications (Chen and Lokhandwala, 1993). The proximal tubular suspension (1 mg protein/ml) was incubated with bromocriptine or forskolin for 15 min at 37°C in a shaking water bath. After incubation, the tubules were permeabilized by rapid freezing in dry ice/acetone and thawing. The reaction mixture in a final volume of 1.025 ml contained (mM): 37.5 imidazole buffer, 70 NaCl, 5 KCl, 1 NaEDTA, 5 $MgCl_2$, 6 NaN_3, 75 Tris-HCl and 100 μl tubular suspension (100 μg protein). The reaction was initiated by the addition of 4 mM ATP. For determination of ouabain-sensitive ATPase, NaCl and KCl were omitted and Tris-HCl (150 mM) and ouabain (1 mM) were added. After incubation at 37°C for 15 min, the reaction was terminated by addition of 50 μl of ice-cold 50% trichloroacetic acid. Samples were centrifuged (3000 rpm) and liberated inorganic phosphate (Pi) in the supernatant was measured as activity of the Na^+,K^+-ATPase. Na^+,K^+-ATPase activity was determined as the difference between total and ouabain-insensitive Na^+,K^+-ATPase activity and was expressed as nmol Pi/mg protein/min.

2.3. cAMP assay

The proximal tubular suspension (7–8 mg/ml protein) was incubated with the agonists such as bromocriptine and forskolin (as indicated in Section 3) for 15 min at 37°C in a shaking water bath similar to the incubating conditions for Na^+,K^+-ATPase. The reaction was terminated by putting the samples in the boiling water bath for 3–5 min. The samples were centrifuged and the supernatant was used for cAMP measurement using a kit (Dupont NEN, Boston, MA, USA). Aliquots of 100 μl of the samples, cAMP-binding protein and [^{3}H]cAMP as the tracer were mixed together and incubated on ice overnight. At the end of incubation, ice-cold charcoal in phosphate-buffered saline was added and the samples were centrifuged. Thus, bound cAMP in the supernatant was separated from free. The supernatant was added to liquid scintillation cocktail, mixed thoroughly and the radioactivity was counted using a β-scintillation counter. The values of cAMP were calculated and expressed as pmol/mg protein using a cAMP standard curve (0.25–8 pmol).

2.4. Data analysis

The values were presented as mean ± S.E.M. and subjected to analysis of variance (one-way ANOVA) for concentration-response curves and to *t*-test for the single concentration responses. $P < 0.05$ was set as the level for statistical significance of the data.

2.5. Materials

Bromocriptine was a gift from Sandoz Pharmaceuticals (E. Hanover, NJ, USA). Forskolin was purchased from Sigma (St. Louis, MO, USA). Pertussis toxin was purchased from Calbiochem Novabiochem (San Diego, CA, USA). The kit for cAMP assay was purchased from DuPont NEN. Other chemicals for various buffers were of the highest purity available and purchased either from Sigma or Fisher Scientific.

3. Results

3.1. Effect of bromocriptine on Na^+,K^+-ATPase activity

Bromocriptine produced a concentration (10^{-9}–10^{-5} M)-dependent stimulation in Na^+,K^+-ATPase activity (Fig. 1A). Significant stimulation was observed at 10 nM of bromocriptine while 1 μM stimulated the enzyme activity maximally (23%). Domperidone (10 mM), a dopamine D_2-like receptor antagonist, significantly antagonized the stimulation of Na^+,K^+-ATPase activity induced by bromocriptine (Fig. 1A). In order to find the role of G protein in this phenomenon, proximal tubules were pretreated with pertussis toxin (200 ng/ml) for 60 min. Pertussis toxin blocked the stimulatory effect of bromocriptine (1 μM) on Na^+,K^+-ATPase activity (Fig. 1B). Pertussis toxin treatment of the tubules did not produce a significant effect on the basal Na^+,K^+-ATPase activity (control 295 ± 30 vs. pertussis toxin treated 283 ± 35 nmol Pi/mg protein per min). A lack of effect of pertussis toxin on basal Na^+,K^+-ATPase activity in the proximal tubules has also been shown earlier by Gurich and Beach (1994).

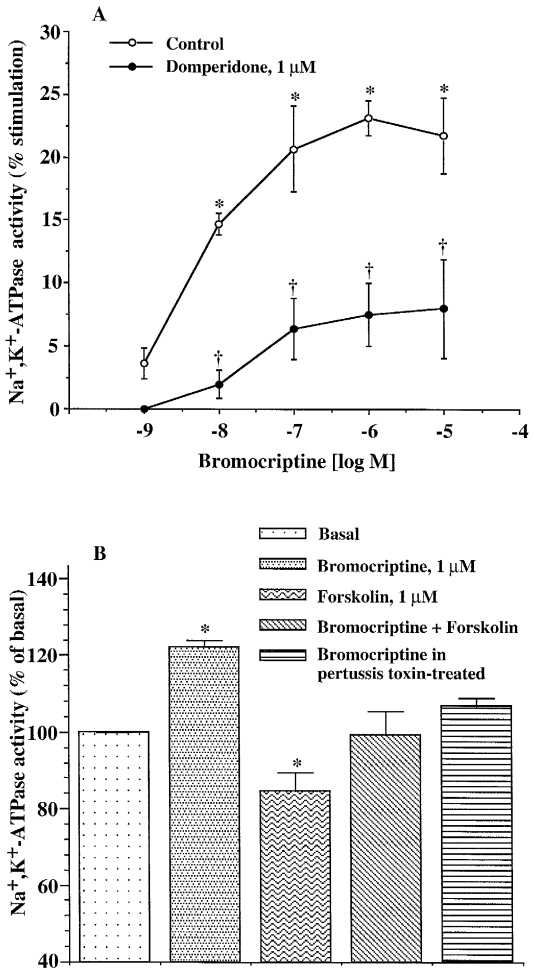

Fig. 1. (A) Effect of bromocriptine (10^{-9}–10^{-5} M) on Na^+,K^+-ATPase activity in proximal tubules in the absence (control) and presence of domperidone (1 μM). The tubules were pretreated with domperidone 10 min prior to the addition of bromocriptine. The basal (without drugs) activity of Na^+,K^+-ATPase was 310 ± 40 nmol Pi/mg protein per min. The values are the mean ± S.E.M. of four experiments performed in triplicates. Data were analyzed by ANOVA. * Significantly different from the basal; † significantly different from respective control value ($P < 0.05$). (B) Effect of bromocriptine (1 μM) and forskolin (1 μM) alone and in combination on Na^+,K^+-ATPase activity. Effect of pertussis toxin (200 ng/ml for 60 min) treatment on bromocriptine-stimulated activity of Na^+,K^+-ATPase is represented by the last bar on the graph. The values are the mean ± S.E.M. of four experiments performed in triplicates. * Significantly different from basal (no drugs); $P < 0.05$. The basal activity in this set of experiments was 295 ± 30 nmol/mg protein per min.

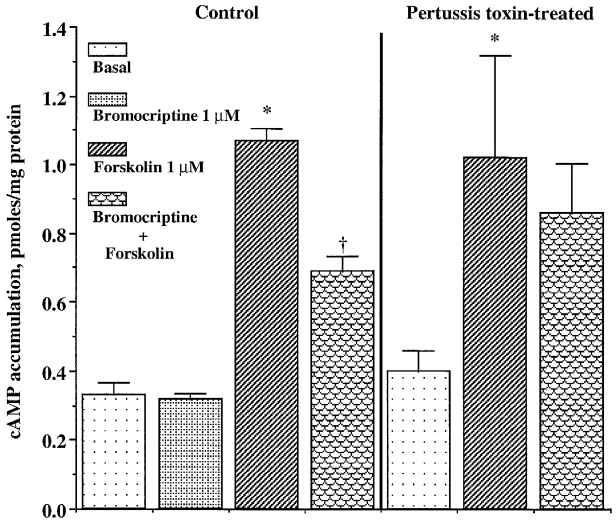

Fig. 2. Effect of bromocriptine (1 μM) on cAMP stimulated by forskolin (1 μM) in control and pertussis toxin (200 ng/ml for 60 min) treated proximal tubules. The values represent the mean ± S.E.M. of four experiments performed in duplicates. * Significantly different from respective basal; † significantly different from forskolin-stimulated cAMP levels; $P < 0.05$. The basal cAMP levels in control and pertussis toxin-treated tubules were 0.33 ± 0.039 and 0.40 ± 0.06 pmol/mg protein, respectively.

3.2. Effect of bromocriptine on cAMP accumulation

Under incubation conditions similar to those for Na^+,K^+-ATPase, the effects of bromocriptine and forskolin were determined on cAMP accumulation in proximal tubular suspension. Bromocriptine (1 μM) alone did not affect the basal level of cAMP accumulation (Fig. 2). However, bromocriptine produced inhibition (32%) in the forskolin (1 μM)-stimulated cAMP levels (Fig. 2). When the proximal tubules were treated with pertussis toxin (200 ng/ml

for 60 min), bromocriptine did not produce a significant inhibition in the forskolin-stimulated cAMP. Forskolin (1 μM) alone stimulated cAMP accumulation approximately by 3-fold in both control and pertussis toxin-treated tubules (Fig. 2). The basal levels of cAMP (pmol/mg protein) in the pertussis toxin-treated proximal tubules (0.40 ± 0.06) was not significantly different compared to the control (0.33 ± 0.039).

4. Discussion

The present study demonstrates the ability of bromocriptine, a dopamine D_2 receptor agonist, to stimulate Na^+,K^+-ATPase activity in the proximal tubules of rat kidney which was antagonized by domperidone, a dopamine D_2 receptor antagonist. This suggested the involvement of dopamine D_2 receptors in the stimulation of Na^+,K^+-ATPase activity. Earlier studies have reported that the activation of dopamine D_2-like receptors alone does not produce any effect on the activity of Na^+,K^+-ATPase (Chen and Lokhandwala, 1993) and if any, dopamine D_2 receptor agonists potentiate the inhibitory effect of dopamine D_1 receptor agonists (Bertorello and Aperia, 1990). However, in a recent report (Yamaguchi et al., 1996) the dopamine D_{2L} receptor was demonstrated to stimulate Na^+,K^+-ATPase activity in murine fibroblast LTK^- cells transfected with rat dopamine D_{2L} receptor cDNA. Furthermore, this study (Yamaguchi et al., 1996) found that the stimulation of Na^+,K^+-ATPase activity by LY171555, a dopamine D_2 agonist, involved Gi protein and the inhibition of adenylyl cyclase. Our study provides results which are in agreement with this report (Yamaguchi et al., 1996) and further extends the observations in the renal proximal tubules. In our study, the stimulation of Na^+,K^+-ATPase by bromocriptine was pertussis toxin-sensitive which suggested the involvement of G protein, likely Gi, in this phenomenon. Moreover, bromocriptine and forskolin counteracted the effects of each other on Na^+,K^+-ATPase activity which may be due to their opposing actions on adenylyl cyclase activity. A similar observation was reported by Yamaguchi et al. (1996) that forskolin-induced inhibition in Na^+,K^+-ATPase activity was blocked by LY171555. This indicated that adenylyl cyclase may be a signaling component which leads to the stimulation of Na^+,K^+-ATPase activity by bromocriptine. The cAMP results partially support these notions. Bromocriptine attenuated the forskolin-induced stimulation of cAMP through a pertussis toxin-sensitive mechanism. We did not find the effect of bromocriptine on the basal levels of cAMP as was reported by Yamaguchi et al. (1996). The reason may be the sensitivity of our cAMP assay. Our assay measures cAMP in pmoles, therefore, a small change may be masked by the high basal contents of cAMP, while the other study (Yamaguchi et al., 1996) used a radioimmunoassay method which measured cAMP in fmoles. Another difference could be the receptor system itself, i.e., rat dopamine D_{2L} receptor expressed in murine fibroblast LTK^- cells versus kidney proximal tubular receptor systems. In an earlier study, dopamine D_2 receptor agonists have also been shown to have no effect on the basal adenylyl cyclase activity in dog kidney proximal tubular membranes (Felder et al., 1989a).

In the presence of bromocriptine and forskolin together, cAMP accumulation did not return to the basal level (Fig. 2) whereas Na^+,K^+-ATPase activity was found to return to the basal level (Fig. 1B). This suggested that the modulation of Na^+,K^+-ATPase activity may not be directly proportional to the cellular level of cAMP. This is also supported by the observation of Yamaguchi et al. (1996) where the alterations in cAMP contents produced by forskolin and LY171555 were not in proportion to the changes in Na^+,K^+-ATPase activity.

As mentioned, earlier reports had demonstrated that dopamine D_2 receptor activation did not affect Na^+,K^+-ATPase activity in the proximal tubules. The reasons for this discrepancy are not known. One difference could be the agonist, bromocriptine, used in this study. The other difference may be the time of incubation of the proximal tubules with the agonist. The effect of bromocriptine on both cAMP accumulation and Na^+,K^+-ATPase activity is a time-sensitive phenomenon (data not shown). The effect of bromocriptine on cAMP lasts only 20 min while the effect on Na^+,K^+-ATPase lasts a little longer, i.e., up to 30 min.

The role of dopamine D_2 receptors in the antidiuretic and antinatriuretic activity of the kidney has been reported earlier. Dopamine D_2 receptor agonists increase renal tubular transport while dopamine D_2-like receptor antagonists produce opposite effects (Siragy et al., 1992, 1990; Laradi et al., 1986). Based on the findings of Yamaguchi et al. (1996) and our observations that dopamine D_2 receptors stimulate Na^+,K^+-ATPase activity, the questions arise whether dopamine D_2 receptors are involved directly in the regulation of tubular Na^+ absorption and whether dopamine D_2 receptors contribute to the basal level of Na^+ transport through the mechanism of the Na^+ pump.

In summary, bromocriptine stimulated Na^+,K^+-ATPase activity by activating dopamine D_2 receptors in the renal proximal tubules. The stimulation of Na^+,K^+-ATPase activity following dopamine D_2 receptor activation may be linked to the inhibition of cAMP through pertussis toxin-sensitive G proteins.

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