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Regulation of Na^+,K^+-ATPase activity by dopamine in cultured rat aortic smooth muscle cells

Sahar M.K. Rashed, Emel Songu-Mize *

Department of Pharmacology and Experimental Therapeutics, Louisiana State University Medical Center, New Orleans, LA, USA

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Abstract

We investigated the effect of dopamine on Na^+,K^+-ATPase activity in cultured aortic smooth muscle cells. Na^+,K^+-ATPase activity was measured by a coupled enzyme assay. Our results demonstrate that dopamine and dopamine receptor agonists, SKF-38393 (a D_1 receptor agonist) and quinpirole (a D_2 receptor agonist) produced 62%, 50% and 49% inhibition of Na^+,K^+-ATPase activity in aortic smooth muscle cells, respectively. The combination of the two agonists produced inhibition similar to that of dopamine. Dopamine- and the agonist-induced Na^+,K^+-ATPase inhibition was blocked by selective receptor antagonists. The Na^+,K^+-ATPase inhibition by SKF-38393 but not by quinpirole was abolished by pertussis toxin. Na^+,K^+-ATPase inhibition was also achieved by guanosine triphosphate analog GTP-γ-S. SKF-38393 but not quinpirole stimulated phosphoinositide hydrolysis rate in rat aortic slices. SKF-38393-induced phosphoinositide hydrolysis stimulation was reversed by SCH-23390, a dopamine D_1 receptor antagonist, and attenuated by pertussis toxin. In conclusion, our observations indicate that dopamine and dopamine receptor agonists inhibit Na^+,K^+-ATPase activity through specific vascular receptors. Dopamine D_1 receptors are linked to pertussis toxin sensitive-mechanism(s) and a GTP-binding protein appears to be coupled to the enzyme inhibition. Finally, the inhibition of Na^+,K^+-ATPase activity in response to dopamine D_1 receptor activation may be mediated by the phospholipase C signaling pathway.

Keywords: Na^+,K^+ATPase, activity; Dopamine; Dopamine receptor; Dopamine signal transduction; Smooth muscle, vascular; Smooth muscle cell, aortic; (Rat)

1. Introduction

Na^+,K^+-ATPase is the enzyme counterpart of the sodium pump which plays an important role in the maintenance of ionic gradients across biological plasma membranes (Fleming, 1980; Mulvany, 1985). These gradients are essential for the intracellular ion concentrations, regulation of cell volume, pH, and also contribute to vascular smooth muscle tone and contractility (Akera and Brody, 1982; Lang and Blaustein, 1980). In the vasculature, inhibition of this enzyme leads to depolarization of the smooth muscle membrane, thereby favoring contraction and increased tone (Hendrickx and Casteels, 1974; Bonaccorsi et al., 1977). Endogenous factors which can regulate the pump in the vascular smooth muscle membranes may play an important role in the resting tone. Regulation of the Na^+,K^+-ATPase or the sodium pump by different factors has been demonstrated by a variety of hormones (Johnson et al., 1986; Gick and Ismail-Beigi, 1990), neurotransmitters (Hernandez-R, 1992; Vizi and Oberfrank, 1992) and other endogenous factors (Hamlyn et al., 1991). Dopamine has been shown to inhibit the Na^+,K^+-ATPase or the sodium pump in the kidney (Bertorello and Aperia, 1990), brain (Bertorello et al., 1990; Felder et al., 1989), and rat tail arteries (Rashed and Songu-Mize, 1995).

We have demonstrated in isolated rat tail arteries that dopamine inhibits the transport function of Na^+,K^+-ATPase, i.e. the sodium pump (Rashed and Songu-Mize, 1995). The sodium pump inhibition in rat tail arteries by dopamine D_1 receptor agonists (SKF-38393) was found to be mediated by the activation of the phospholipase C system (Rashed and Songu-Mize, 1995). The purpose of this study was to investigate the effect of dopamine on the sodium pump at a cellular level in the vascular system. Therefore, we studied the effect of dopamine on the Na^+,K^+-ATPase, the enzyme counterpart of the sodium pump, in cultured aortic smooth muscle cells. In addition,

* Corresponding author. Louisiana State University Medical Center, Department of Pharmacology and Experimental Therapeutics, 1901 Perdido Street, Rm #7107, New Orleans, LA 70112, USA. Tel.: (504)-568-4740; fax: (504)-568-2361.

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studies were performed to identify the elements involved in the signaling pathway(s) mediating the inhibition of vascular Na^+,K^+-ATPase by dopamine through dopamine D_1 and D_2 receptors.

2. Materials and methods

2.1. Preparation of cultured aortic smooth muscle cells

Rat aortic smooth muscle cells were prepared as described before (Songu-Mize et al., 1990). Briefly, rat aortas were isolated and cleaned with phosphate buffer saline several times. The arteries were cut into small pieces and treated with 200 U/ml collagenase type I, for 30 min at 37°C. This was followed by a second enzyme digestion with 15 U/ml of elastase III and 200 U/ml of collagenase I for 2–3 h at 37°C. Following the second enzyme treatment, cells were washed extensively with M199 medium and centrifuged at 500 × g for 5 min at room temperature. Cells were harvested and cultured in the medium containing 20% fetal bovine serum for five days before transferring to a medium containig 10% serum. The efficiency of the technique and purity of the smooth muscle cells were tested. The cells were positively identified as smooth muscle cells by indirect immunofluorescent staining for α-actin antibody and anti-mouse IgG fluorescein isothiocyanate conjugate. Experiments were performed on confluent quiescent aortic smooth muscle cells between the passages 3 and 7.

2.2. Measurement of Na^+,K^+-ATPase activity in aortic smooth muscle cells

The enzyme activity was measured by using a coupled enzyme assay method (Nørby, 1988). Aortic smooth muscle cells were grown on cover slips and were treated with the drugs as described in each experiment and immediately permeabilized by freezing the cells for 10 min at −20°C. Cover slips were cut into two halves, and added to a cuvette containing a reaction mixture, with and without 2 mM ouabain. The reaction mixture contained (in mM) KCl – 20, NaCl – 100, $MgSO_4.7H_2O$ – 8.0, EGTA – 0.5, Tris – 40, phospho-enol pyruvate – 10, NADH – 0.25, fructose-1,6-diphosphate – 1.0, ATP – 5, and lactate dehydrogenase – 1.1 U/ml, pyruvate kinase – 0.9 U/ml. The cuvettes containing cover slips were placed in a spectrophotometer, and the absorbance readings at 340 nm at 37°C were taken at one minute intervals. The slope of the disappearance curve of NADH represents the ATP hydrolysis rate. To obtain the Na^+,K^+-ATPase activity, the slope of the activity in the presence of ouabain (ouabain-resistant ATPase activity) was subtracted from the slope obtained in the absence of ouabain (total ATPase activity). Cell protein was determined by Lowry method (Lowry et al., 1951) and the activity was expressed as nmol/mg cell protein/min. Bovine serum albumin was used as a standard.

2.3. Measurement of phosphoinositide hydrolysis rate

Rat aortas were freshly pooled from 3–5 male rats and phosphoinositide hydrolysis rate was measured (Gonzales and Crews, 1985). Rat aortas were washed with oxygenated Krebs-Ringer bicarbonate buffer (KRB, composition in mM: NaCl – 118, KCl – 4.7, $CaCl_2$ – 0.75, KH_2PO_4 – 1.18, $MgSO_4$ – 1.18, $NaHCO_3$ – 24.8, glucose – 10; pH 7.4, 37°C), and sliced using a MacIlwain tissue chopper set at 350 μm. Sliced tissues were washed twice with KRB and incubated with 20 μCi of 0.3 μM [^{3}H]*myo*-inositol for 60 min. Labeling was terminated by removing and washing the tissue slices 3 times with KRB.

Tissue slices were distributed according to different treatments and incubated with drugs for 60 min in KRB containing 10 mM LiCl isotonically substituted for NaCl. Drug treatments were terminated by adding 1.0 ml of chloroform/methanol (1 : 2 v/v), 0.35 ml chloroform, and 0.35 ml deionized water.

Samples were centrifuged for 10 min at 900 × g, and aliquots of the [^{3}H]inositol metabolites were collected from the aqueous phase to be separated by ion exchange chromatography. The bound [^{3}H]inositol phosphates were eluted from Dowex 1-X8 (formate form) using 1.0 M ammonium formate/0.1 M formic acid and the radioactivity was measured using a scintillation counter. Another aliquot from the lipid phase was removed, air dried, and measured for radioactivity. Data were expressed as the ratio of [^{3}H]inositol phosphate metabolites (dpms in the aqueous phase) to the total [^{3}H]inositol incorporated (dpms in aqueous + lipid phases).

2.4. Pertussis toxin preparation

In experiments where pertussis toxin was used, the toxin was activated by 1.25 mM ATP and 1.25 mM dithiothreitol, at 37°C for 30 min before adding to the aortic smooth muscle cells.

2.5. Statistical analyses

We used an analysis of variance (ANOVA) followed by Fisher's least significant difference or Scheffé's test to determine the significant differences among treatments. Student's *t*-test was also used when appropriate and a P value of ≤ 0.05 was considered significant.

2.6. Drugs

Collagenase, elastase and trypsin inhibitor were obtained from Sigma Chemical Company (St. Louis, MO, USA). Dopamine hydrochloride, *R*(+)-SCH-23390 HCl (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5,-tetrahydro-1*H*-3-benzazepine-7,8-diol hydrochloride), *S*(−)-sulpiride, *R*(+)-SKF-38393 HCl (*R*(+)-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diol hydrochloride),

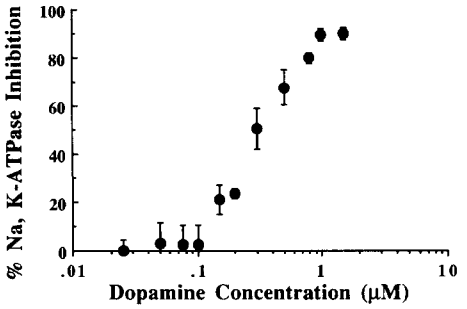

Fig. 1. Effect of dopamine on Na^+,K^+-ATPase activity of aortic smooth muscle cells in culture. Cells were incubated for 10 min with dopamine. The Na^+,K^+-ATPase activity was measured as described in the methods. Values are means ± S.E. ($n = 5$).

(−)-quinpirole HCl, pertussis toxin, phentolamine, propranolol, haloperidol, U-73122, and phorbol 12-myristate 13-acetate (PMA) were obtained from Research Biochemicals (Natick, MA, USA). ^{86}RbCl and [^{3}H]inositol were obtained from NEN Chemical Company (Boston MA, USA). Dopamine, dopamine receptor agonists and antagonists were dissolved in Krebs containing ascorbic acid (0.1% w/v) to prevent oxidation. Ascorbic acid was added to all of the control samples as a vehicle.

3. Results

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

Dopamine produced a significant inhibition of Na^+,K^+-ATPase activity in a dose dependent manner (Fig. 1). The control Na^+,K^+-ATPase activity was 194 ± 19 nmol/mg protein/min. Dopamine produced a 67% inhibition of the Na^+,K^+-ATPase activity at 0.5 μM concentration (63 ± 12 nmol/mg protein/min). We chose the 0.5 μM concentration in subsequent experiments. The ouabain-insensitive ATPase activity was not affected by any of the dopamine concentrations used (46 ± 8 nmol/mg protein/min, $n = 10$). Phentolamine (1 μM), an α-adrenoceptor antagonist, and propranolol (1 μM), a β-receptor antagonist did not alter dopamine inhibtion of the Na^+,K^+-ATPase in the aortic smooth muscle cells (data not shown).

3.2. Effect of dopamine and dopamine receptor agonists on Na^+,K^+-ATPase activity

We determined the effect of dopamine and dopamine receptor agonists, SKF-38393 and quinpirole, on Na^+,K^+-ATPase activity (Fig. 2). The basal activity of the Na^+,K^+-ATPase in the aortic smooth muscle cells was 182 ± 16 nmol/mg protein/min. Dopamine produced a 62% inhibition of the Na^+,K^+-ATPase activity. The dopamine D_1 receptor agonist SKF-38393 and the dopamine D_2 receptor agonist quinpirole both at a concentration of 0.5 μM produced a 50% and 49% inhibition, respectively. The combination of the two agonists produced a 58% inhibition of the enzyme activity (Fig. 2). The ouabain-resistant ATPase activity was not affected by the drug treatments and the mean value was 40 ± 7 nmol/mg protein/min ($n = 15$).

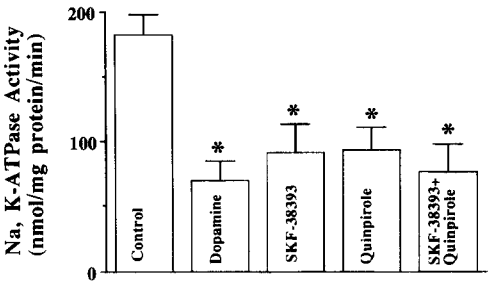

Fig. 2. Effect of dopamine and dopamine receptor agonists on Na^+,K^+-ATPase activity. The bar graph represents the Na^+,K^+-ATPase activity of cultured aortic smooth muscle cells, treated with vehicle (control, $n = 22$), dopamine ($n = 11$), dopamine D_1 receptor agonist SKF-38393 ($n = 14$), dopamine D_2 receptor agonist quinpirole ($n = 16$), and a combination of dopamine D_1 and D_2 receptor agonists ($n = 12$). All drug concentrations were 0.5 μM. Values are the least square means ± S.E. Data are analyzed by one-way ANOVA, followed by Fisher's least significant difference test. * Significant difference between the control and the drug treatments, $P \leq 0.05$.

3.3. Effect of dopamine receptor antagonists on dopamine inhibition of Na^+,K^+-ATPase activity

To investigate the presence of specific dopamine D_1 and D_2 receptors by which dopamine produces inhibition of the Na^+,K^+-ATPase activity, the cells were incubated with receptor antagonists SCH-23390 and sulpiride (0.5 μM each) prior to treatment with dopamine (Fig. 3). The basal activity of Na^+,K^+-ATPase was 191 ± 19 nmol/mg

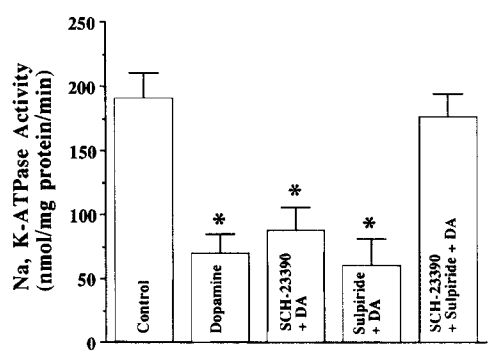

Fig. 3. Effect of dopamine receptor antagonists on dopamine inhibition of Na^+,K^+-ATPase activity. The bar graph represents the Na^+,K^+-ATPase activity of cultured aortic smooth muscle cells treated with vehicle (control), dopamine, dopamine D_1 receptor antagonist SCH-23390 plus dopamine (DA), dopamine D_2 receptor antagonist sulpiride plus dopamine and a combination of SCH-23390, sulpiride plus dopamine, $n = 6$ for each group. All drug concentrations were 0.5 μM. Values are the least square means ± S.E. Data are analyzed by one-way ANOVA, followed by Fisher's least significant difference test. * Significant difference between the control and drug treatments, $P \leq 0.05$.

protein/min. Dopamine produced a 63% inhibition of the Na^+,K^+-ATPase. In the presence of the dopamine D_1 receptor antagonist SCH-23390 and the dopamine D_2 receptor antagonist sulpiride, dopamine produced a 54% and a 68% inhibition of the Na^+,K^+-ATPase activity, respectively (Fig. 3). The combination of the two receptor antagonists (0.5 μM each) resulted in a complete reversal of the dopamine effect. Haloperidol (1 μM), a nonselective dopamine receptor antagonist, blocked dopamine inhibition of Na^+,K^+-ATPase (data not shown).

Additional experiments were performed to assure that SKF-38393 and quinpirole inhibition of Na^+,K^+-ATPase activity were dose-dependent and the choice of a 0.5 μM concentration for agonist experiments was appropriate in this system. The results are shown in Table 1. There was a dose-dependent inhibition, and the 0.5 μM dose produced approximately 50 percent inhibition with both drugs. Therefore, 0.5 μM concentration was used in the subsequent agonist experiments.

3.4. Effect of dopamine receptor antagonists on dopamine receptor agonist inhibition of Na^+,K^+-ATPase

Experiments were designed as above except that dopamine was replaced by the corresponding receptor agonists (Fig. 4). The basal activity of Na^+,K^+-ATPase was 178 ± 19 nmol/mg protein/min. SKF-38393 produced a 62% inhibition of Na^+,K^+-ATPase activity. This effect of SKF-38393 was blocked in the presence of SCH-23390 (0.5 μM). Quinpirole produced a 59% inhibition. This inhibition was also blocked in the presence of sulpiride (0.5 μM). In incubations where the two dopamine receptor antagonists, D_1 and D_2 (0.5 μM each) were added simultaneously prior to the D_1 and D_2 receptor agonists, the Na^+,K^+-ATPase inhibition by the agonists was not observed (Fig. 4). The receptor antagonists SCH-23390 or sulpiride alone did not affect the basal Na^+,K^+-ATPase activity significantly (168 ± 10 and 180 ± 12 nmol/mg protein/min, respectively, $n = 6$).

We performed additional experiments to elucidate the effect of D_2 receptor antagonist on the D_1 receptor agonist, and conversely the D_1 receptor antagonist on D_2 receptor agonist. The control activity of the enzyme was 293 ± 30 nmol/mg protein/min ($n = 4$). The inclusion of the D_2 receptor antagonist sulpiride in the presence of the D_1 receptor agonist SKF-38393 did not affect the inhibitory effect of the latter (101 ± 16 nmol/mg protein/min ($n = 4$), a 65% inhibition compared to the control). Likewise, the presence of the D_1 receptor antagonist SCH-23389 with the D_2 receptor agonist quinpirol did not not affect the inhibitory effect of the latter (92 ± 10 nmol/mg protein/min ($n = 4$), a 69% inhibition compared to the control).

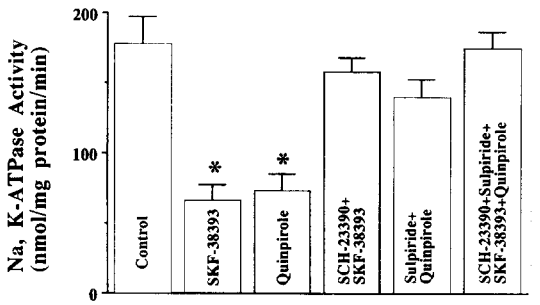

Fig. 4. Effect of dopamine receptor antagonists on dopamine receptor agonist inhibition of Na^+,K^+-ATPase. The bar graph represents the Na^+,K^+-ATPase activity of cultured aortic smooth muscle cells treated with vehicle (control, $n = 6$). SKF-38393 ($n = 6$), quinpirole ($n = 7$), SCH-23390 plus SKF-38393 ($n = 3$), sulpiride plus quinpirole ($n = 3$) and combination of the antagonists and agonists ($n = 3$). All drug concentrations were 0.5 μM. Values are the least square means $\pm$ S.E. Data are analyzed by one-way ANOVA, followed by Fisher's least significant difference test. * Significant difference between the control and drug treatments, $P \leq 0.05$.

3.5. Effect of pertussis toxin on dopamine and dopamine receptor agonist inhibition of Na^+,K^+-ATPase activity

Experiments were performed to identify the signaling mechanism(s) which may be involved in dopamine inhibition of Na^+,K^+-ATPase activity in the aortic smooth muscle cells. We tested the sensitivity of the signaling cascade involved in dopamine inhibition of Na^+,K^+-ATPase to pertussis toxin. The basal Na^+,K^+-ATPase activity was 186 ± 20 nmol/mg protein/min (Fig. 5). SKF-38393 produced a 65% inhibition of Na^+,K^+-ATPase activity. This inhibition was abolished in the presence of pertussis toxin (100 ng/ml). Quinpirole produced a 56% inhibition of Na^+,K^+-ATPase activity. However, pertussis toxin pretreatment did not affect inhibition produced by quinpirole. Pertussis toxin alone did not affect the basal Na^+,K^+-ATPase activity (171 ± 4.0 nmol/mg protein/min).

3.6. Effect of GTP-γ-S on Na^+,K^+-ATPase activity

To investigate the presence of a GTP-binding protein linked to Na^+,K^+-ATPase inhibition, the non-hydrolyzable

Table 1
Percent inhibition of Na^+,K^+-ATPase by dopamine receptor agonists

Receptor agonist	Drug concentration (μM)		
	0.25	0.50	1.00
SKF-38393	44	52	64
	(281 ± 54)	(241 ± 53)	(180 ± 12)
Quinpirole	31	60	80
	(345 ± 26)	(198 ± 29)	(101 ± 18)

Values represent percent inhibition of Na^+,K^+-ATPase compared to the control. The control value was 501 ± 32 ($n = 5$). Numbers in parentheses are actual values for enzyme activities expressed as nmol/mg protein/min. There was a significant inhibition ($P \leq 0.05$) by agonists at each concentration used; ANOVA and Scheffé's F-test were applied, $n = 4$ for each experimental group.

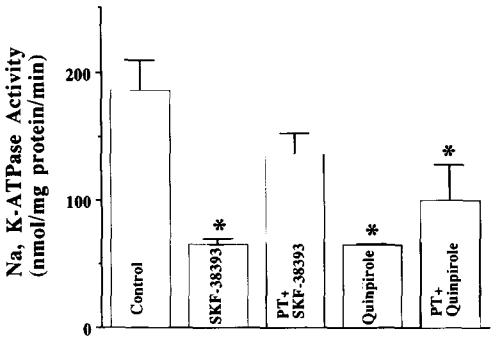

Fig. 5. Effect of pertussis toxin on dopamine receptor agonist inhibition of Na^+,K^+-ATPase activity. The bar graph represents the Na^+,K^+-ATPase activity of cultured aortic smooth muscle cells treated with vehicle (control $n = 9$), SKF-38393 ($n = 5$), SKF-38393 plus pertussis toxin (100 ng/ml, $n = 5$), quinpirole ($n = 5$), quinpirole plus pertussis toxin ($n = 5$). All agonist concentrations were 0.5 μM. Values are the least square means ± S.E. Data are analyzed by one-way ANOVA, followed by Fisher's least significant difference test. * Significant difference between the control and drug treatments, $P \leq 0.05$.

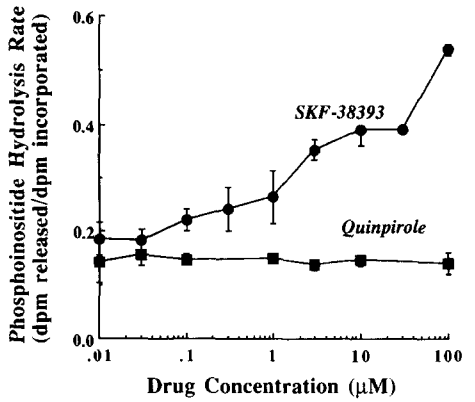

Fig. 7. Effect of dopamine receptor agonists on phosphoinositide hydrolysis in rat aortic slices. The line graph represents the effect of increasing concentrations of dopamine D_1 receptor agonist SKF-38393 (circles), and dopamine D_2 receptor agonist, quinpirole (squares), on phosphoinositide hydrolysis rate. Rat aortic slices were incubated with the drugs for 60 min following the labeling period as described in Methods. Values are means ± S.E. ($n = 6$).

analog of GTP, GTP-γ-S (guanosine-5′-*O*-3-thiophosphate) was used. Different concentrations of GTP-γ-S produced Na^+,K^+-ATPase inhibition in a dose-dependent manner (Fig. 6). The control activity was 117 ± 18 nmol/mg protein/min (Fig. 6). With 10 μM of GTP-γ-S, Na^+,K^+-ATPase activity was reduced significantly by 36%. 15 μM of GTP-γ-S produced a 72% inhibition of Na^+,K^+-ATPase activity.

3.7. Effect of dopamine receptor agonists on phosphoinositide hydrolysis

To investigate the involvement of phospholipase C system in mediating dopamine signaling in rat aorta, experiments were designed to measure the phosphoinositide hydrolysis rate in response to dopamine receptor agonist in freshly isolated rat aortas. Data presented in Fig. 7 demonstrate a dose-dependent stimulation of the phosphoinositide hydrolysis rate produced by SKF-38393. The control phosphoinositide hydrolysis rate was 0.189 ± 0.01 (dpms released/dpms incorporated). Quinpirole did not affect the phosphoinositide hydrolysis rate at any concentration used (Fig. 7). Additional experiments were performed using U-73122, a phospholipase C inhibitor, and phorbol 12-myristate 13-acetate (PMA), a protein kinase C activator. U-73122 (1 μM) reversed 35% of dopamine inhibition of Na^+,K^+-ATPase activity. PMA (1 μM) treatment for 60 seconds produced a 45% inhibition of the Na^+,K^+-ATPase activity.

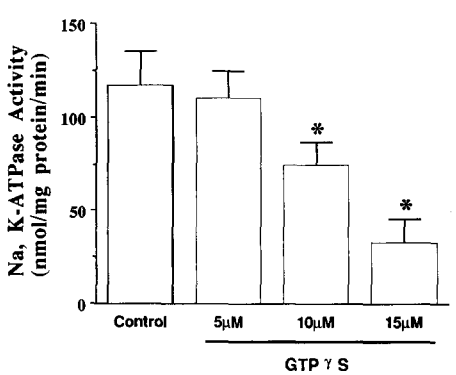

Fig. 6. Effect of GTP-γ-S on Na^+,K^+-ATPase activity. The bar graph represents inhibition of the Na^+,K^+-ATPase activity in cultured aortic smooth muscle cells treated with different concentrations of guanosine-5′-*O*-(3-thiophosphate) (GTP-γ-S) as indicated. Values are the least square means ± S.E. Data are analyzed by one-way ANOVA, followed by Fisher's least significant difference test. * Significant difference between the control and drug treatments, $P \leq 0.05$.

3.8. Effect of dopamine D_1 receptor antagonist on SKF-38393-stimulation of phosphoinositide hydrolysis

To demonstrate that phosphoinositide hydrolysis in response to SKF-38393 is due to a selective interaction with the dopamine D_1 receptor, experiments were performed using a selective dopamine D_1 receptor antagonist, SCH-23390. Aortic slices were preincubated with 10 μM of SCH-23390 for 15 min before the addition of different concentrations of SKF-38393. SCH-23390 blocked the phosphoinositide stimulation produced by SKF-38393 (Fig. 8).

3.9. Effect of pertussis toxin on SKF-38393 stimulation of phosphoinositide hydrolysis

To demonstrate the coupling of phospholipase C system with the dopamine D_1 receptors, experiments were performed where the rat aortic slices were incubated with pertussis toxin (100 μg/ml) for 1 h prior to the addition of the receptor agonist. The basal phosphoinositide hydrolysis rate was 0.05 ± 0.01 dpms released/dpms incorpo-

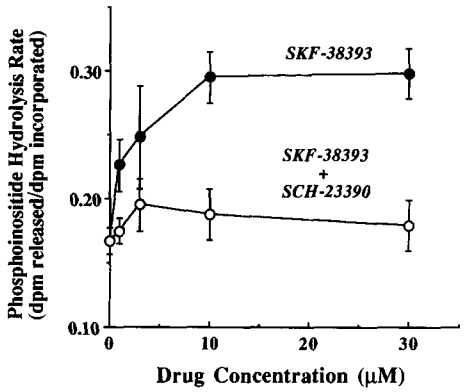

Fig. 8. Effect of D_1 receptor antagonist on SKF-38393-mediated stimulation of phosphoinositide hydrolysis rate in rat aortic slices. The line graph represents the effect of increasing concentrations of dopamine D_1 receptor agonist SKF-38393 on phosphoinositide hydrolysis rate in the absence (solid circles) and in the presence (open circles) of dopamine D_1 receptor antagonist SCH-23390. SCH-23390 was added to tissue slices 15 min before the addition of SKF-38393. Values are means ± S.E. ($n = 6$).

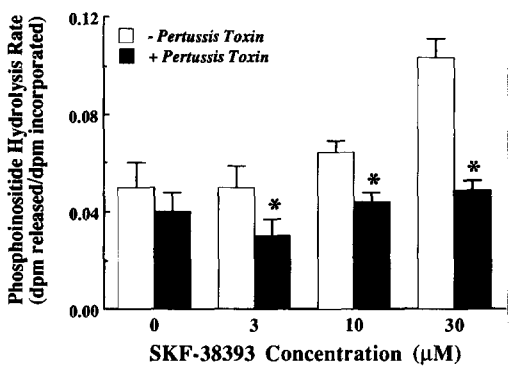

Fig. 9. Effect of pertussis toxin on D_1 receptor agonist-stimulated phosphoinositide hydrolysis rate in rat aortic slices. The bar graph represents the effect of pertussis toxin (100 ng/ml) on phosphoinositide hydrolysis stimulated by SKF-38393. Tissue slices were exposed to pertussis toxin for 60 min during the labeling period with [^{3}H]*myo*-inositol. The rate of hydrolysis was measured after an additional 60 min incubation of the tissues with different concentrations of SKF-38393. Results were analyzed using one-way ANOVA followed by Fisher's least significant difference test. Values are least square means ± S.E. ($n = 4$). * Significant difference between tissues treated with SKF-38393 without (striped bars) and with pertussis toxin (solid bars), $P \leq 0.05$.

rated (Fig. 9). Pertussis toxin alone did not affect the basal phosphoinositide hydrolysis (0.04 ± 0.01 dpms released/dpms incorporated). SKF-38393 at concentrations of 3, 10 and 30 μM stimulated the phosphoinositide hydrolysis rate. In the presence of pertussis toxin this effect of SKF-38393 was attenuated by 40, 31 and 52%, respectively (Fig. 9).

4. Discussion

In this study we investigated the regulation of the vascular Na^+,K^+ -ATPase activity by dopamine, and identified some of the signaling mechanisms involved in this action. Our results indicated the presence of dopamine receptor subtypes, D_1 and D_2 in rat vascular smooth muscle cells. In addition, we demonstrated that dopamine inhibits the cellular Na^+,K^+-ATPase through the activation of dopamine D_1 and D_2 receptor subtypes. This finding complements our recent previous findings in isolated rat tail arteries (Rashed and Songu-Mize, 1995). In that study, we demonstrated that the functional counterpart of the enzyme Na^+,K^+-ATPase, the sodium pump, was significantly inhibited through the activation of dopamine D_1 and D_2 receptors. In addition, dopamine produced an increase in perfusion pressure when the arteries were perfused in an in vitro constant-flow system, suggesting a functional role for dopamine in the vascular system. In the present study we showed, at a cellular level, that the Na^+,K^+-ATPase inhibition through the two receptor subtypes was not additive. This finding led us to pursue the identification of the signal transduction mechanism(s) mediating each vascular receptor type utilizing the cultured cell system.

The isolated cultured cell system provides some advantages over isolated tissue preparation. First, this system allows us to measure the biochemical counterpart of the sodium pump, the activity of Na^+,K^+-ATPase. Indeed, we were able to confirm our previous findings, that is, in rat arterial system the sodium pump activity is inhibited by dopamine (Rashed and Songu-Mize, 1995) by demonstrating a significant inhibition in the corresponding enzyme activity. Second, in an isolated tissue preparation other cell types, such as the fibroblasts and endothelial cells, may also participate in overall respnse to dopamine. And third, since there are no nerve endings in cultured cell preparation, the interaction of the post and presynaptic receptors does not occur, thereby allowing a more simplified and clear assesment of the cellular mechanisms underlying dopamine effect.

Dopamine regulation of Na^+,K^+-ATPase activity has been implicated in a variety of other tissues. For example, in rat kidney and the cells of the neurostriatum dopamine inhibits the Na^+,K^+-ATPase through the activation of D_1 and D_2 receptors (Bertorello and Aperia, 1988, 1990; Bertorello et al., 1990) and this effect was additive in the neostriatal cells (Bertorello et al., 1990). However, there appears to be a difference in the mechanism of dopamine inhibition in the vascular smooth muscle cells. Although, both receptor subtypes, D_1 and D_2, participated in Na^+,K^+-ATPase inhibition, we found that this inhibition was not additive in the vasculature. This finding was confirmed by the use of selective antagonist, SCH-23390 and sulpiride. Yet in another tissue system, isolated renal proximal tubules, prior activation of dopamine D_1 receptor appeared to play a permissive role in D_2 inhibition of the Na^+,K^+-ATPase, suggesting an interaction of the receptors in inhibition of this enzyme (Bertorello and Aperia, 1990). The non-additive nature of the Na^+,K^+-ATPase inhibition in our system may be explained by an interaction between

the two subtypes of dopamine receptors, a possibility which is not addressed within the focus of this study.

The second messenger systems linked to dopamine receptors in different tissues display considerable variability (Jose et al., 1991). Different laboratories reported alternate signal transduction pathways even within the same tissue. For example, Felder et al. (1989) reported that in renal tubular membranes dopamine D_1 receptor activation is linked to the stimulation of both the phopholipase C and adenylate cyclase systems. Whereas, Bertorello and Aperia (1990) reported that the D_1 receptor in rat proximal tubules is linked to the adenylate cyclase system only. The details of the signal transduction pathways linked to vascular dopamine receptors have not been worked out.

We found that the inhibition of the Na^+,K^+-ATPase in aortic smooth muscle cells through the activation of dopamine D_1 but not the D_2 receptors is a pertussis toxin-sensitive event and is linked to the phospholipase C system. This finding is demonstrated by the elevation of phosphoinositide hydrolysis rate after activation of the D_1 receptors, and its block by a selective D_1 receptor blocker (Figs. 7 and 8). It was clearly demonstrated in our system that the dopamine D_2 receptor activation does not produce these effects. This effect of D_1 receptor activation on phosphoinositide hydrolysis was pertussis toxin-sensitive (Fig. 9).

Additional evidence for the involvement of phospholipase C system in dopamine-induced Na^+,K^+-ATPase inhibition is provided by the U-73122 (a phospholipase C inhibitor) and PMA (a protein kinase C activator) studies. U-73122 partially reversed dopamine inhibition of Na^+,K^+-ATPase, whereas PMA inhibited the enzyme.

Our data in cultured aortic smooth muscle cells agree with a number of studies which demonstrated the involvement of dopamine D_1 receptors in the activation of phospholipase C by dopamine in the renal tissue (Felder et al., 1989; Jose et al., 1991). Others have demonstrated in kidney proximal tubules that dopamine-induced Na^+,K^+-ATPase inhibition is through a pertussis toxin mechanism as well (Bertorello and Aperia, 1988). Our findings of elevated phosphoinositide hydrolysis rate in aortic smooth muscle cells, taken together with the findings that this was a pertussis toxin-sensitive event, the dopamine inhibition of Na^+,K^+-ATPase was reversed by U-73122, and PMA inhibited the enzyme activity, imply that dopamine inhibition of Na^+,K^+-ATPase is through the activation of the phospholipase C system. Additional support for our conclusion can be driven from the findings of Simmons et al. (1986), which indicate that the inhibition of Na^+,K^+-ATPase activity is a result of decreased levels of *myo*-inositol (a substrate for phosphoinositide hydrolysis reaction) in rat aortic intima. This conclusion does not eliminate the possibility of the interaction of dopamine D_1 and D_2 receptors with the adenylate cyclase system which has been also shown to be linked to the inhibition of Na^+,K^+-ATPase activity in the renal tubular cells (Aperia et al., 1991). Additionally, inhibition of Na^+,K^+-ATPase activity by dopamine D_1 receptor in the cortical collecting duct was found to be mediated through the stimulation of phospholipase-A_2 and arachidonic acid release (Satoh et al., 1992).

Activation of the phospholipase C system is known to stimulate the generation of diacyl glycerol and inositol trisphosphate which are responsible for the activation of protein kinase C and the increase of the cytosolic levels of calcium, respectively (Berridge, 1989; Abdel-Latif, 1986). Protein kinase C and other proteins such as calmodulin and calnaktin, increase the sensitivity of the sodium pump/Na^+,K^+-ATPase to the physiological concentrations of intracellular Ca^{2+} (Yingst, 1988) or the enzyme phosphorylation (Bertorello and Aperia, 1989). Additionally, stimulation of the adenylate cyclase system and thus the activation of protein kinase A is suggested to play a role in Na^+,K^+-ATPase inhibition through increasing the levels of the phosphorylated form of the enzyme (Bertorello and Aperia, 1990). Activation of these kinases in blood vessels could be one of the mechanisms by which Na^+,K^+-ATPase is inhibited. The increased levels of Ca^{2+} in aortas (Jelicks and Gupta, 1990), and elevated inositol trisphosphate binding capacity in vascular smooth muscle cells (Bernier and Guillemette, 1993) isolated from spontaneously hypertensive rats have been demonstrated. The increase in the aortic Ca^{2+} levels may contribute to the increases in the blood vessel's contractility and elevation of the blood pressure. In addition, phospholipase C activity was found to be high in the aortic wall of the spontaneously hypertensive rats (Uehara et al., 1988).

Since dopamine receptors belong to the seven transmembrane receptor family, they are likely to be coupled to GTP-binding proteins (Johnson and Friedman, 1993). The possible involvement of a GTP-binding protein in the process of Na^+,K^+-ATPase inhibition is addressed in experiments described in Fig. 8. Data from these experiments provide evidence for a GTP-binding protein as an element in the cascade of Na^+,K^+-ATPase inhibition. A nonhydrolyzable analog of GTP, GTP-γ-S, inhibited the Na^+,K^+-ATPase activity in a dose-dependent manner.

In conclusion, our data demonstrated that in rat vascular smooth muscle cells the inhibition of Na^+,K^+-ATPase by dopamine is mediated by both the dopamine D_1 and the dopamine D_2 receptor subtypes. Dopamine D_1 receptor-mediated enzyme inhibition is linked to the phospholipase C system through a pertussis toxin-sensitive process. Furthermore, the activation of a G-protein is associated with vascular Na^+,K^+-ATPase inhibition.

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