ELSEVIER

European Journal of Pharmacology 284 (1995) 289–297

ejp

Regulation of Na^+-pump activity by dopamine in rat tail arteries ☆

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

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

Received 19 January 1995; revised 7 June 1995; accepted 13 June 1995

Abstract

We investigated the effect of dopamine on the vascular Na^+-pump activity in isolated rat tail artery sections. Effect of dopamine on vascular tone was also assessed using a perfused tail artery preparation. Dopamine inhibited the Na^+-pump activity in isolated rat tail arteries in a dose-dependent manner. Both SKF-38393 HCl, a selective dopamine D_1 receptor agonist, and quinpirole HCl, a selective dopamine D_2 receptor agonist inhibited the Na^+-pump activity. The inhibition of the Na^+-pump by dopamine was accompanied with a transient increase in the vascular tone. SKF-38393, but not quinpirole produced a sustained increase in the vascular tone. Tissues preincubated simultaneously with SCH-23390 HCl, a selective dopamine D_1 receptor antagonist, and sulpiride, a selective dopamine D_2 receptor antagonist, prevented the dopamine inhibition of the Na^+-pump activity. Pertussis toxin blocked the Na^+-pump inhibition produced by the dopamine D_1 receptor agonist but not by the dopamine D_2 agonist. Similarly, the dopamine D_1 receptor but not dopamine D_2 agonist increased the rate of phosphoinositide hydrolysis in rat tail artery sections. Our results indicate that dopamine inhibits rat tail artery Na^+-pump activity, and both the dopamine D_1 receptor and dopamine D_2 receptor subtypes mediate this effect. It appears that dopamine D_1 receptor, but not dopamine D_2 receptor agonist-induced inhibition of the Na^+-pump is mediated by a pertussis toxin-sensitive mechanism and may be coupled to the activation of the phospholipase C system in rat tail arteries. The modulation of the Na^+-pump by dopamine may contribute to the vascular tone.

Keywords: Na^+-pump; Dopamine receptor; Phosphoinositide hydrolysis; Pertussis toxin; Vascular tone

1. Introduction

The Na^+-pump, or the Na^+,K^+-ATPase, plays an important role in the maintenance of ionic gradients across the cell membranes (Skou and Esmann, 1992; De Weer, 1985). These gradients constitute the electrical potential of the plasma membrane, which in turn regulates intracellular ion concentrations, pH, cell volume, and contributes to vascular smooth muscle tone and contractility (Mulvany, 1985; Lang and Blaustein, 1980). Na^+,K^+-ATPase is regulated by several factors such as intracellular sodium, extracellular potassium concentrations, adenosine triphosphate and the cardiac glycosides (Akera and Brody, 1982). The activity of this enzyme can also be regulated by hormones (Middleron et al., 1990) and neurotransmitters (Hernandez, 1992; Vizi and Oberfrank, 1992). Catecholamines such as epinephrine and norepinephrine have been shown to modulate the Na^+-pump activity by activating the adrenoceptors (Hernandez, 1992). Dopamine has been implicated as an endogenous inhibitor of Na^+,K^+-ATPase in renal proximal convoluted tubules (Bertorello and Aperia, 1990) and in the isolated striatal neurons (Bertorello et al., 1990).

In the kidney, dopamine binds to its receptors, dopamine D_1 and D_2 (Jose et al., 1991; Goldberg et al., 1978) and produces natriuresis (Yuasa et al., 1992). However, in the heart, dopamine acts as an inotropic agent and is used in certain cases of congestive heart failure. The mechanism of dopamine to produce inotropy is through its adrenergic effect in the heart and by activation of the adenylate cyclase system (Goldberg, 1975; Brodde, 1990)

Different studies demonstrated the involvement of either adenylate cyclase or phospholipase C (PLC)

* Corresponding author. 1901 Perdido Street, Room No. 7108, Department of Pharmacology and Experimental Therapeutics, Louisiana State University Medical Center, New Orleans LA 70112, USA. Tel. (504) 568-4740, fax (504) 568-2361.

☆ Supported by the American Heart Association, Louisiana, Inc., Postdoctoral Fellowship and a grant from NIH, HL-32270.

Elsevier Science B.V.
SSDI 0014-2999(95)00363-0

signaling pathways in dopamine D_1- and D_2-mediated effects in a variety of tissues. In renal tubules both systems are activated independently in response to dopamine D_1 receptor agonist SKF-38393 (Felder et al., 1989). In rat renal cortical membranes and the brain, dopamine and dopamine D_1 receptor agonists activate the PLC system (Felder et al., 1989; and Mahan, 1990). In proximal tubule segments, stimulation of the dopamine D_2 receptors appears to activate the PLC pathway. However, this process is dependent on the activation of the adenylate cyclase system by dopamine D_1 receptor agonists (Bertorello et al., 1990).

Dopamine receptor subtypes have been characterized in rat blood vessels such as the cerebral and mesenteric arteries (Creese and Sibley, 1979; Amenta et al., 1990), and human heart (Amenta et al., 1993), using radioligand binding and autoradiographic techniques. However the functional significance of dopamine receptors and the signaling pathways involved are not identified in blood vessels. In preliminary reports we have demonstrated an inhibitory effect of dopamine on Na^+-pump activity in isolated rat tail arteries and cultured aortic smooth muscle cells (Rashed and Songu-Mize 1991, 1993a,b). In the present study we are reporting the relative participation of dopamine D_1 and D_2 receptors to produce Na^+-pump inhibition in rat tail arteries and the relevant functional effects on the vascular tone. In addition, we investigated the signaling events involved in Na^+-pump inhibition by dopamine and its receptor agonists.

2. Materials and methods

2.1. Measurement of Na^+-pump activity in rat tail arteries

Ouabain-sensitive $^{86}Rb^+$-uptake technique was used for measuring the Na^+-pump activity in freshly isolated rat tail arteries as described before (Songu-Mize, 1991) Male Sprague-Dawley rats (Harlan, Indianapolis, IN) of 250–300 g were used. Rats were anesthetized with chloralhydrate (300 mg/kg) supplemented with xylazine (10 mg/kg) in accordance with the institutional guidelines. Rat tail arteries were isolated and pooled from seven to ten rats, and then cleaned in Krebs-Henseleit bicarbonate buffer (pH 7.4, bubbled with $CO_2$5%-$O_2$95%, composition in mM: $NaHCO_3$ 27.2, NaCl 119, $NaH_2PO_4 \cdot H_2O$ 1, $MgSO_4 \cdot H_2O$ 1.2, $CaCl_2 \cdot 2H_2O$ 2.5, dextrose 11, KCl 5). Rat tail arteries were cut into 1-inch pieces and incubated on ice for 20 min in K^+-free Krebs buffer (six to ten pieces per treatment). This procedure allows sodium accumulation in the tissue and maximizes the Na^+-pump activity (Songu-Mize, 1991). Tissue pieces were then transferred to incubation tubes containing Krebs buffer at 37°C containing 5 mM KCl. Drugs were added and incubated with the tissues for 10 min. $^{86}RbCl$ ($\approx 10^6$ CPM/tube, 10–50 nM) was added to start the uptake reaction. ^{86}Rb was used as a tracer for K^+ (Smith et al., 1986). The uptake of $^{86}Rb^+$ (and K^+) was terminated at the end of 15 min. The rate of ouabain sensitive ^{86}Rb-uptake (specific uptake) was linear up to 40 min (Songu-Mize, 1991; Songu-Mize et al., 1987). The ouabain-insensitive $^{86}Rb^+$-uptake was determined in the presence of 2 mM ouabain. Following the uptake, tissues were washed of excess radioactivity and blotted dry. The radioactivity was measured using a gamma counter. The dry weights of the tail artery pieces were recorded. The Na^+-pump activity (ouabain sensitive $^{86}Rb^+$-uptake) was calculated as the total $^{86}Rb^+$-uptake minus the ouabain-insensitive $^{86}Rb^+$-uptake. The activity is expressed as nmol ($^{86}Rb^+ + K^+$)/mg tissue dry weight/15 min.

2.2. Measurement of phosphoinositide hydrolysis rate

Rat tail arteries were freshly pooled from three to five male rats and phosphoinositide hydrolysis rate was measured (Gonzales and Crews, 1985). In this procedure, rat tail arteries 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). Tissues were sliced using a Macllwain tissue chopper set at 350 μm. Sliced tissues were washed twice with KRB and incubated with 20 μCi of 0.3 μM myo-[^{3}H]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 $900 \times g$, 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 are expressed as the ratio of [^{3}H]inositol phosphate metabolites released (dpm in the aqueous phase) to the total [^{3}H]inositol incorporated (dpm in the aqueous phase + dpm in the lipid phase).

2.3. Pertussis toxin preparation

In experiments where pertussis toxin was used, the toxin was activated by 1.25 mM ATP and 1.25 mM

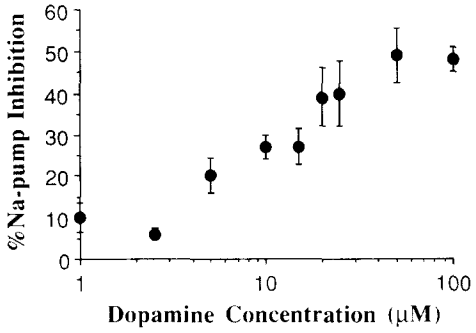

Fig. 1. Effect of dopamine on Na^+-pump activity. Isolated rat tail artery pieces were incubated for 10 min with the indicated concentrations of dopamine and the Na^+-pump activity was determined as described in Methods. Values are means ± S.E. For each point four to five tissue pieces were used.

dithiothreitol, at 37°C for 30 min before adding to the tissues.

2.4. Measurement of the tail artery perfusion pressure

Rats were anesthetized with sodium pentobarbital, 35 mg/kg, i.p., and received 100 U of heparin. A 1-cm strip of the tail artery was isolated near the base of the tail and cannulated using stretched PE-50 (0.58 mm) polyethylene tubing. The tail was severed from the body and placed in a tissue bath containing oxygenated (95% O_2-5% CO_2) Krebs-Henseleit bicarbonate buffer with 30 μM EDTA. The tail artery was perfused in situ with the same buffer by using a peristaltic pump (Ismatec) at a constant flow of 2.5 ml/min. After a 30–45 min stabilization period the baseline perfusion pressure was measured by using a pressure transducer (COBE CDX) attached to the arterial cannula and recorded on a polygraphic recorder (Grass Model 7D). Bolus doses of dopamine and the dopamine agonists were then injected and the developed pressure was recorded. Baseline or near-baseline pressures were achieved after each drug before administering the next drug. Since a constant flow was maintained the changes in the perfusion pressure represent the changes in vascular resistance.

2.5. Statistical analysis

We used an analysis of variance (ANOVA) followed by Fisher's least significant difference 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. Chemicals

Dopamine hydrochloride, pertussis toxin, $R(+)$-SCH-23390 HCl ($R(+)$-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride), $S(-)$-sulpiride, $R(+)$-SKF-38393 HCl ($R(+)$-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrochloride), (−)-quinpirole HCl, phentolamine, propranolol, haloperidol, U-73122 and phorbol 12-myristate 13-acetate (PMA) were supplied from Research Biochemical, Natick, MA, USA. ^{86}RbCl and [^{3}H]inositol were obtained from NEN Chemical Company, Boston MA. Sphingosine and phenylephrine was obtained from Sigma chemical company. Dopamine, dopamine agonists and antagonists were dissolved in Krebs containing ascorbic acid (0.1% w/v) to prevent oxidation.

3. Results

3.1. Effect of dopamine and dopamine receptor agonists on Na^+-pump activity

Dopamine produced a dose-dependent inhibition of ouabain-sensitive $^{86}Rb^+$ uptake in isolated rat tail arteries (Fig. 1). We selected 10 μM dopamine for our subsequent experiments at which concentration, a 30% inhibition of the pump was observed. Ouabain-re-

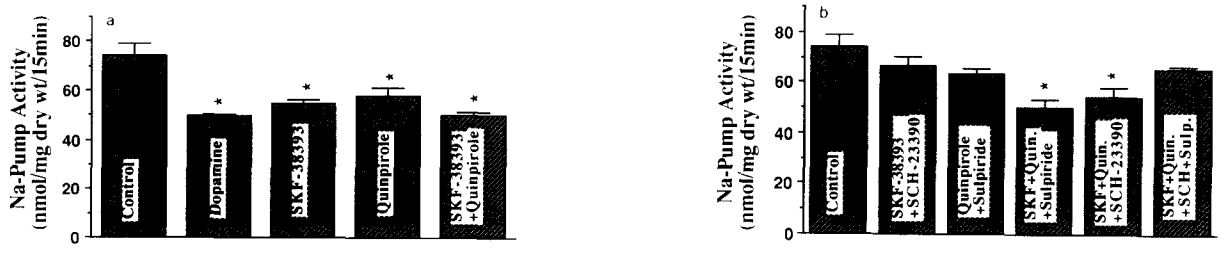

Fig. 2. Effect of dopamine and dopamine receptor agonists on Na^+-pump activity. The bar graph represents the Na^+-pump activity ($^{86}Rb^+$-K^+) in rat tail arteries treated with vehicle (control, $n = 7$), dopamine ($n = 8$), dopamine D_1 receptor agonist, SKF-38393 ($n = 7$), dopamine D_2 receptor agonist, quinpirole ($n = 7$) and a combination of dopamine D_1 and D_2 receptor agonists ($n = 6$) (2a) and vehicle (the same control as 2a, $n = 7$), SKF-38393 plus SCH-23390 ($n = 7$), quinpirole plus sulpiride ($n = 7$), SKF-38393 and quinpirole plus sulpiride ($n = 7$), SKF-38393 and quinpirole plus SCH-23390 ($n = 7$) and SKF-38393 and quinpirole plus SCH-23390 and sulpiride ($n = 7$) (2b). All drug concentrations were 10 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, $P \leq 0.05$.

sistant uptake of ($^{86}Rb^+ + K^+$) was not affected at any of the dopamine concentrations used (16.1 ± 1.7 nmol/mg dry weight per 15 min).

To determine whether one or both of the dopamine receptor subtypes contribute to the inhibition of the vascular Na^+-pump, we tested the effect of different receptor agonists on Na^+-pump activity. Basal (control) Na^+-pump activity (total $^{86}Rb^+$-uptake minus ouabain-insensitive $^{86}Rb^+$-uptake) in rat tail arteries was 74.0 ± 2.9 nmol/mg dry weight per 15 min (Fig. 2a). Dopamine (10 μM) inhibited the Na^+-pump activity significantly by 33%. The dopamine D_1 receptor agonist, SKF-38393 (10 μM), and the dopamine D_2 receptor agonist, quinpirole (10 μM), produced a 27% and a 22% inhibition of the Na^+-pump activity, respectively, compared to the control. The combination of the two dopamine receptor agonists inhibited the Na^+-pump activity by 32%. The ouabain-insensitive Na^+-pump activity was not affected by the drug treatments and the mean value was 15.7 ± 1.8 nmol/mg dry weight per 15 min ($n = 17$).

To eliminate the possibility that dopamine may be exerting its effect by acting through adrenoceptors, we pretreated the tissues with phentolamine, an α-adrenoceptor antagonist, or propranolol, a β-adrenoceptor antagonist. Neither phentolamine (1 μM), nor propranolol (1 μM) had any effect on the inhibitory action of dopamine on the pump activity (data not shown).

3.2. Effect of dopamine receptor antagonists on dopamine-inhibition of Na^+-pump activity

To confirm the involvement of the specific dopaminergic receptor subtypes, we performed experiments using the selective receptor agonists with their respective antagonists. Rat tail arteries were preincubated with SCH-23390 HCl, a selective dopamine D_1 receptor antagonist, and sulpiride, a selective dopamine D_2 receptor antagonist 10 μM each, for 10 min prior to the addition of SKF-38393 and quinpirole. The results are shown in (Fig. 2b). SCH-23390 prevented the inhibition produced by SKF-38393. Similarly, sulpiride prevented inhibition of the Na^+-pump activity by quinpirole (see Fig. 2a,b). When a combination of dopamine D_1 and D_2 receptor agonists, SKF-38393 and quinpirole, were used neither sulpiride nor SCH-23390 alone could block the inhibitory effect of the agonists. However, the combination of the two antagonists completely blocked the combined effect of the two agonists (Fig. 2b). The ouabain-insensitive uptake was not affected by the drug treatments and the mean value was 16.0 ± 1.2 nmol/mg dry weight per 15 min ($n = 26$).

Additional experiments with receptor antagonists were performed to confirm the involvement of dopamine D_1 and D_2 receptors in dopamine action (Fig. 3). Dopamine produced a 29% inhibition of the

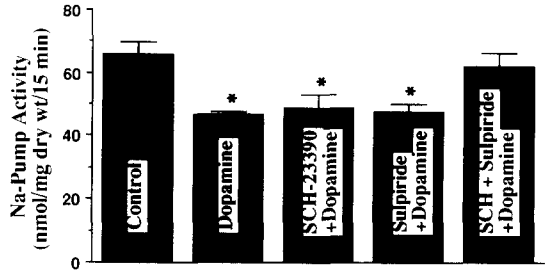

Fig. 3. Effect of dopamine receptor antagonists on dopamine inhibition of Na^+-pump activity. The bar graph represents the Na^+-pump activity in rat tail arteries treated with vehicle (control, $n = 6$), dopamine ($n = 5$), dopamine D_1 receptor antagonist SCH-23390 plus dopamine ($n = 6$), dopamine D_2 receptor antagonist sulpiride plus dopamine ($n = 5$), and a combination of SCH-23390 and sulpiride plus dopamine ($n = 4$). All drug concentrations were 10 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, $P \leq 0.05$.

Na^+-pump activity. When the tissues were pretreated with SCH-23390 or sulpiride, dopamine inhibition was not affected. However, when the tissues were pretreated with the combination of the two receptor antagonists dopamine inhibition of Na^+-pump activity was blocked (Fig. 3). These data indicate that each receptor participates in the effect of dopamine on Na^+-pump. We also used a non-selective dopamine antagonist, haloperidol, to confirm the participation of the two receptors in Na^+-pump inhibition. Haloperidol completely prevented the inhibitory action of dopamine on the pump.

3.3. Effect of pertussis toxin on dopamine and dopamine receptor agonist-inhibition of Na^+-pump activity

Experiments were performed to characterize the signaling mechanism(s) which may be involved in dopamine effect on Na^+-pump activity in rat tail arteries. We tested the sensitivity of the system to pertussis toxin. Rat tail artery pieces were pretreated with pertussis toxin, 50 ng/ml, for 30 min at 37°C. In the absence of pertussis toxin, dopamine (10 μM) produced a 45% inhibition of the Na^+-pump activity (Table 1). In the presence of pertussis toxin, dopamine did not affect the Na^+-pump activity. Similarly, SKF-39383 produced a 30% inhibition of the Na^+-pump activity and this inhibition was abolished in the presence of pertussis toxin. In contrast, a 25% inhibition produced by quinpirole was not affected by the prior treatment with pertussis toxin. Pertussis toxin alone did not change the basal Na^+-pump activity. The ouabain-insensitive Na^+-pump activity was not affected by the drug treatments and the mean value was 14.3 ± 0.4 nmol/mg dry weight per 15 min ($n = 31$) (Table 1).

Table 1
Effect of pertussis toxin on dopamine receptor agonist-inhibition of Na^+-pump activity

	Control	DA	DA + PT	DA1	DA1 + PT	DA2	DA2 + PT
Total	57.6 ± 3.2	39.3 ± 4.1	52.9 ± 2.5	44.8 ± 1.2	51.0 ± 2.2	46.8 ± 1.4	45.4 ± 2.0
+ Ouabain	17.8 ± 0.8	16.5 ± 1.8	18.6 ± 2.1	15.7 ± 2.0	14.0 ± 1.4	15.7 ± 0.7	13.6 ± 1.5
Na^+-pump	41.3 ± 3.2	22.8 ± 4.1	34.3 ± 2.5	29.1 ± 1.2	37.0 ± 2.2	31.1 ± 1.4	32.0 ± 2.0
n	7	5	5	6	6	6	6
% inhibition		45	16	30	10	25	23
Significance, *P*		≤ 0.05	n.s.	≤ 0.05	n.s.	≤ 0.05	≤ 0.05

Isolated rat tail arteries were pretreated for 30 min at 37°C with vehicle or pertussis toxin (PT, 50 ng/ml), followed by incubation with dopamine (DA), dopamine D_1 (DA1) receptor agonist SKF-38393, or dopamine D_2 (DA2) receptor agonist quinpirole for 10 min (10 μM each) and the Na^+-pump activity was determined as described in Materials and methods. Values are the least square means ± S.E. Units are nmol (^{86}Rb + K) taken up/mg dry weight/15 min. Total: total (^{86}Rb + K) uptake; + Ouabain: uptake in the presence of 2 mM ouabain; Na^+-pump: total uptake minus '+ Ouabain' uptake; % inhibition: Na^+-pump inhibition by the drugs; *n*: number of tissue pieces. Significance: *P* values were obtained by one-way ANOVA followed by Fisher's least significant difference test and indicate differences between the control and treatment values.

3.4. Effect of dopamine receptor agonists on phosphoinositide hydrolysis

To investigate the involvement of the phospholipase C system in dopamine signal transduction in mediating Na^+-pump inhibition, tail artery pieces were incubated with different concentrations of dopamine and dopamine receptor agonists, SKF-38393 and quinpirole (Fig. 4). Dopamine and SKF-38393 increased phosphoinositide hydrolysis rate in a dose-dependent manner, and maximum increase was observed at 30 μM concentration. Quinpirole did not increase phosphoinositide hydrolysis significantly at any concentration. Basal phosphoinositide hydrolysis rate ratio was 0.172 ± 0.02 (dpm released/dpm incorporated, $n = 6$).

To provide evidence for the specific interaction of a dopamine D1 receptor agonist with a specific vascular dopamine D_1 receptor to induce phosphoinositide hydrolysis, tissues were preincubated with a dopamine D_1 receptor antagonist SCH-23390 (10 μM) for 15 min prior to the addition of dopamine D_1 receptor agonist SKF-38393. SKF-38393-induced phosphoinositide hydrolysis was prevented by SCH-23390 at all concentrations (Fig. 5). SCH-23390, at concentrations 0.1, 3, 30 and 100 μM, did not affect the basal phosphoinositide-hydrolysis rate (data not shown).

To further demonstrate the coupling between dopamine D_1 receptors and phospholipase C system, rat tail artery pieces were preincubated with pertussis toxin (100 μg/ml) for 1 h prior to the addition of SKF-38393. SKF-38393 stimulated phosphoinositide hydrolysis rate in a dose-dependent manner (Fig. 6). At all concentrations of SKF-38393, pertussis toxin produced a significant attenuation of phosphoinositide hydrolysis rate (Fig. 6). The maximal effect of pertussis toxin on SKF-stimulated phosphoinositide hydrolysis

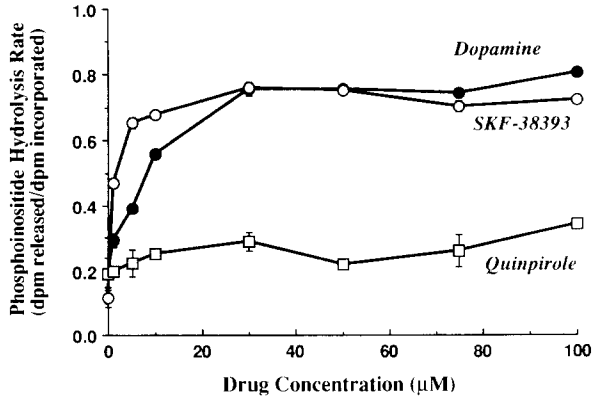

Fig. 4. Effect of dopamine and dopamine receptor agonists on phosphoinositide hydrolysis. The line graph represents the effect of increasing concentrations of dopamine, dopamine D_1 receptor agonist, SKF-38393, and dopamine D_2 receptor agonist, quinpirole, on phosphoinositide hydrolysis rate. Rat tail artery slices were incubated with the drugs for 60 min following the labelling period as described in methods. Values are means ± S.E. ($n = 3$).

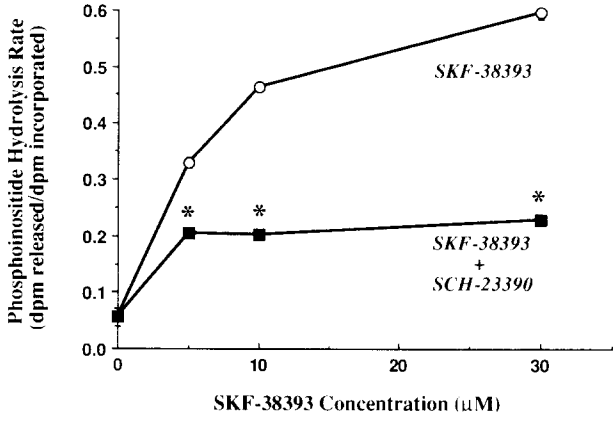

Fig. 5. Effect of dopamine D_1 receptor antagonist on phosphoinositide hydrolysis rate stimulated by SKF-38393. The line graph represents the effect of increasing concentrations of dopamine D_1 receptor agonist SKF-38393 on phosphoinositide hydrolysis rate in the absence (white circles) and in the presence (black squares) of dopamine D_1 receptor antagonist, SCH-23390. SCH-23390 was added to tissue slices 15 min before the addition 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. ($n = 3$). * Significant difference between tissues pretreated with and without SCH-23390.

rate was observed at 0.4 μM SKF-38393 concentration. Pertussis toxin alone did not change the basal phosphoinositide hydrolysis rate (Fig. 6). After demonstrating that phospholipase C system stimulation is coupled to dopamine D_1 receptor activation in rat tail arteries, we preformed experiments to investigate the link between the phospholipase C activation and dopamine-induced Na^+-pump inhibition. U-73122, a phospholipase C inhibitor (10 μM) prevented dopamine induced Na^+-pump inhibition in rat tail arteries (45% inhibition by dopamine vs. 15% in the presence of U-73122. In addition, activation of protein kinase C by phorbol 12-myristate 13-acetate, PMA (1 μM) produced 67% inhibition of the pump activity. Sphingosine (1 μM), a protein kinase C inhibitor reduced dopamine inhibition of the Na^+-pump activity by a 50% compared with dopamine alone. In these experiments, U-73122 and shingosine did not affect the basal levels of the pump activity (72 ± 4 and 71 ± 5, respectively, vs. control 74 ± 3 nmol/mg dry weight per 15 min ($n = 5$). These findings support our observation of the involvement of the phospholipase C system in the events leading to the arterial pump inhibition.

3.5. Effect of dopamine and dopamine receptor agonists on vascular tone

To determine the physiological consequence of dopamine-inhibition of the Na^+-pump activity, dopamine and dopamine receptor agonists were administered into the perfused rat tail artery. Bolus injections of dopamine produced transient increases in the basal perfusion pressure in a dose-dependent manner; dopamine doses of 0.2, 0.5, 1.0 and 2.0 μg (4, 10, 20 and 40 μM, respectively) produced 50, 70, 110 and 155 mmHg increases above the basal perfusion pressure, respectively (Fig. 7, panel A). Bolus injections of SKF-38393 (0.6, 1.5, 3, 6 μg or 1.6, 4, 20, 40 μM, respectively) produced a sustained increase in the perfusion pressure (Fig. 7, panel B). Quinpirole did not affect the perfusion pressure at any of the doses used (0.15, 0.3, 0.75, 1.5, 3 μg or 2, 4, 10, 20, 40 μM, respectively, Fig. 7, panel C). However, 1.5 μg (2.4 μM) and 30 ng (0.4 μM) bolus doses of quinpirole reversed the sustained increase in the perfusion pressure produced by SKF-38393 (0.1 μM) (Fig. 7, panel D). Phenylephrine was used as a positive control which produced a dose-dependent increase in the perfusion pressure (Fig. 7, panel C). To eliminate the possibility that dopamine effect may be due to enhanced release of catecholamines from the nerve terminals (Burke et al., 1995; Sun and Reis, 1994; Patil et al., 1994; Kirkpatrick and Burnstock, 1994), two bolus injections of 100 μM tyramine was applied before dopamine administration. Tyramine by itself did not change the perfusion pressure in these preparations. Moreover, tyramine treatment did not alter the effect of dopamine or the agonists.

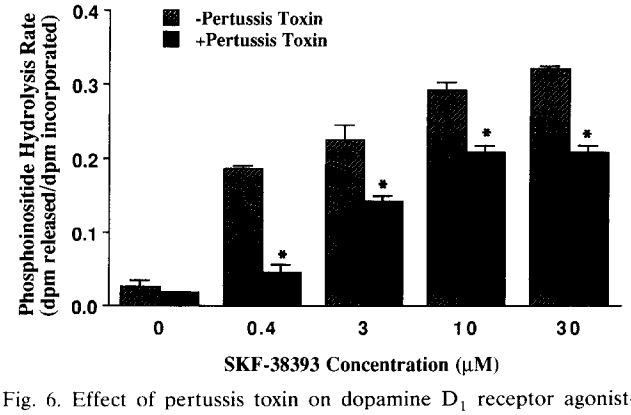

Fig. 6. Effect of pertussis toxin on dopamine D_1 receptor agonist-stimulated phosphoinositide hydrolysis rate. The bar graph represents the effect of pertussis toxin (100 ng/ml) on dopamine D_1 receptor agonist SKF-38393-induced phosphoinositide hydrolysis. Tissue slices were exposed to pertussis toxin 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. ($n = 3$). * Significant difference between tissues treated with SKF-38393 (hatched bars) and SKF-38393 plus pertussis toxin (solid bars).

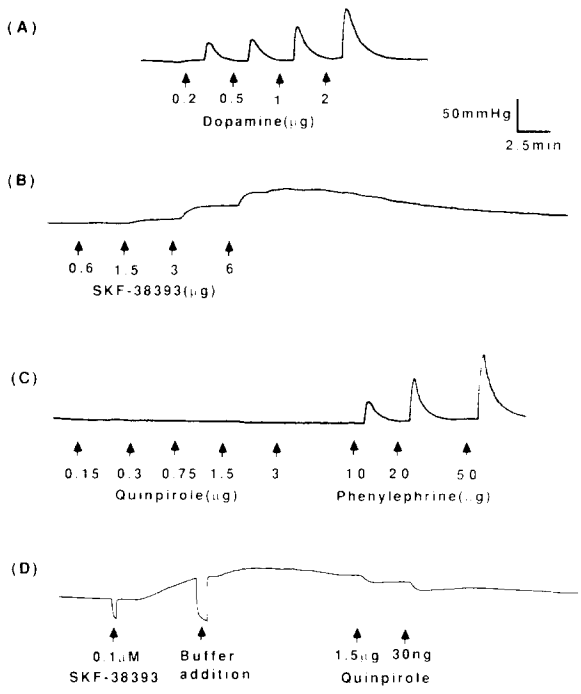

Fig. 7. Effect of dopamine and dopamine agonists on rat tail artery perfusion pressure. Isolated tail arteries were perfused as described in Methods. A representative pressure tracing demonstrates the effect of bolus doses of dopamine (A), SKF-38393 (B), quinpirole and phenylephrine (C) and a constant perfusion of SKF-38393 (0.1 μM) and bolus injections of quinpirole (D) on perfusion pressure. 15 min equilibration period were allowed between drugs administrations. Similar results were obtained in three other experiments.

4. Discussion

The regulation of Na^+,K^+-ATPase activity by dopamine in a variety of tissues implicated the importance of this neurotransmitter in balancing the ionic gradients across plasma membranes. In the renal and the central nervous systems dopamine inhibits the Na^+,K^+-ATPase through the activation of the dopamine D_1 and D_2 receptor subtypes (Bertorello and Aperia 1988, 1990; Bertorello et al., 1990). Our study was conducted to evaluate the effect of dopamine on vascular Na^+-pump activity and identify the signaling mechanisms involved in dopamine action in blood vessels. As has been demonstrated in the rat kidney proximal tubules Bertorello and Aperia (1990) and the striatal neurons (Bertorello et al., 1990) dopamine inhibited the Na^+-pump activity significantly in the isolated rat tail arteries. This is the first demonstration of the regulation of the functional aspect of the Na^+,K^+-ATPase, the Na^+-pump, by dopamine in the vasculature.

Our results demonstrate that dopamine inhibits the vascular Na^+-pump through the activation of both the dopamine D_1 and D_2 receptors. The simultaneous application of the two receptor agonists did not produce an additive effect in this system (Fig. 2a). Experiments with selective antagonists, SCH-23390 and sulpiride, confirmed the non-additive effects of dopamine D_1 and D_2 receptor activation on Na^+-pump inhibition (Fig. 3). This is unlike the findings in the neurons of the neurostriatum where the inhibition of Na^+,K^+-ATPase in response to dopamine D_1 and D_2 receptor activation was additive (Bertorello et al., 1990). Moreover, in the renal proximal tubules, prior activation of dopamine D_1 receptor appears to play a permissive role in D_2-inhibition of the Na^+,K^+-ATPase (Bertorello and Aperia, 1990) suggesting the dependency of D_2 receptor subtypes on the activation of dopamine D_1 receptor to produce Na^+,K^+-ATPase inhibition. In our hands, both the dopamine D_1 and D_2 receptor activation appeared to inhibit the Na^+-pump in a non-additive manner. The non-additive nature of the Na^+-pump inhibition through these receptors may be explained by an interaction between the dopamine receptors, a possibility which is not addressed in this study.

There is considerable variability in the second messenger systems linked to dopamine receptors in different tissues (Jose et al., 1991). Even within the same tissue, different laboratories reported alternate signal transduction pathways linked to dopamine receptors. For example, in rat proximal tubules, Bertorello and Aperia 1990, reported that dopamine D_1 receptors are linked to the adenylate cyclase activation, whereas dopamine D_2 receptors are linked to the phospholipase C system. They also suggest that activation of the phospholipase C system by dopamine D_2 is dependent on dopamine D_1 receptor activation (Bertorello and Aperia, 1990). In contrast, Felder et al. (1989), reported that dopamine D_1 receptor activation is linked to the stimulation of both the phospholipase C and adenylate cyclase systems independently in renal tubular membranes. However, in the vasculature the details of the signal transduction pathways linked to dopamine receptors have not been worked out.

Our results in rat tail arteries showed that dopamine D_1 receptor stimulation is linked to the phospholipase C system demonstrated by the elevation of phosphoinositide hydrolysis rate (Fig. 4). This effect on phosphoinositide hydrolysis was reversed by a selective dopamine D_1 receptor antagonist and was a pertussis toxin-sensitive event (Figs. 5 and 6). In addition, inhibition of the Na^+-pump activity mediated by dopamine D_1 receptors was also a pertussis toxin-sensitive event (Table 1). Therefore, it is likely that Na^+-pump inhibition by dopamine D_1 receptor activation is coupled to the phospholipase C system. The involvement or the interaction of other signaling system(s) such as adenylate cyclase is a possibility.

Additional evidence for the involvement of phospholipase C system in dopamine-induced Na^+-pump inhibition was provided by using U-73122, a phospholipase C inhibitor, PMA a protein kinase C activator and sphingosine, a protein kinase C inhibitor. Both U-73122 and sphingosine attenuated dopamine-inhibition of the Na^+-pump. PMA produced a significant inhibition of the Na^+-pump activity. Therefore, it is possible that protein kinase C, directly through the phosphorylation of the Na^+-pump or indirectly through its effect on Na^+-influx, may produce Na^+-pump inhibition. Interestingly, the degree of reversal of dopamine inhibition of the pump by U-73122 was greater than the one observed by sphingosine. This difference may be due to the fact that U-73122, which uncouples G-protein from phospholipase C, is also an inhibitor of phospholipase C and phospholipase A_2. This compound can also decrease cytosolic free Ca^{2+} by lowering IP_3 level. Therefore, the U-73122 affects more than one component in the phospholipase C signaling system cascade. However, sphingosine inhibits the phospholipase C system at the level of protein kinase C only. The differences of the effects of these compounds suggest the participation of phospholipase A_2 activation and/or cellular free Ca^{2+} increase in dopamine actions in the vasculature.

The possible involvement of a GTP-binding protein in the process of Na^+-pump inhibition is addressed in a study utilizing cultured aortic smooth muscle cells (Rashed and Songu-Mize, 1993). In that study a non-hydrolyzable analog of GTP, GTP-γ-S, inhibited the Na^+,K^+-ATPase activity. Since the inhibition of Na^+-pump activity by dopamine in rat tail arteries was also

found to be sensitive to pertussis toxin, the presence of a G-protein in the signaling cascade is implicated in this artery.

The functional role of dopamine in the kidney appears to be the regulation of natriuresis and diuresis by inhibiting Na^+,K^+-ATPase and Na^+-H^+ antiport and thus contribution to Na^+ retention (Aperia et al., 1991). In hypertension, dopamine-regulation of renal Na^+,K^+-ATPase appears to be altered. In both SHR and the Dahl salt-sensitive strain of hypertensive rats dopamine fails to inhibit renal Na^+,K^+-ATPase (Chen et al., 1993; Nishi et al., 1993). In the SHR the defect is likely to involve dopamine D_1-phospholipase C coupling (Chen et al., 1993). whereas in Dahl salt-sensitive rats, dopamine D_1-adenylate cyclase coupling appears to be involved (Nishi et al., 1993). In concert with this finding Dahl salt sensitive rats displayed attenuated natriuretic response to dopamine (Nishi et al., 1993).

In vascular smooth muscle cells contraction-relaxation is regulated by the level of intracellular free Ca^{2+} concentration (Bevan, 1993). Na^+-pump contributes to the maintenance of the cellular membrane potential (Thomas, 1972; Fleming, 1980). Therefore, the pump may play a role in the mechanisms affecting vascular tone and contractility. Inhibition of the Na^+-pump in the vasculartur causes depolarization and contraction and/or increased sensitivity to endogenous vasoconstrictor agents (Blaustein; 1977; Hamlyn et al., 1991). Very small changes in cellular membrane potential of only a few mV may result in prominent contraction or relaxation of the vascular smooth muscle (Hermsmeyer, 1976). We have shown in a tail artery constant-flow perfusion system that dopamine increases the perfusion pressure which is an indicator of vascular resistance (Fig. 7). This is in accord with the observed inhibition of the Na^+-pump by dopamine in the isolated tail artery sections. Interestingly, a dopamine D_1 receptor agonist produced a sustained increase in perfusion pressure, whereas the dopamine D_2 receptor agonist had no effect when administered alone. However, the dopamine D_2 receptor agonist reversed the sustained elevation obtained by the dopamine D_1 receptor agonist, suggesting differential but interactive effects of the two receptor subtypes on the functional consequence of dopamine action in the vasculature. We interpret the net transient effect of dopamine to be a summation of the activation of the two receptor subtypes with opposing effect with different second messenger signaling systems.

Although, the inhibition of the vascular Na^+-pump by dopamine, and contraction of the vasculature are associated events, they do not *necessarily* constitute a cause-effect relationship. It is possible that the D_1 receptor agonist produces a sustained contraction of the tail artery though a mechanism independent of Na^+-pump inhibition.

In conclusion, our data demonstrated that dopamine inhibits the Na^+-pump activity in rat tail arteries through the activation of dopamine D_1 and D_2 receptor subtypes. This inhibition of the enzyme may contribute to the observed increase in the tail artery perfusion pressure. The Na^+-pump inhibition produced by dopamine D_1 receptor activation is mediated by a pertussis toxin-sensitive mechanism, and the stimulation of the phospholipase C system may be involved in this process.

References

Akera T. and T.M. Brody, 1982. Myocardial membranes: regulation and function of the sodium pump, Ann. Rev. Physiol. 44, 375.

Amenta, F., W.L. Collier and A. Ricci, 1990, Autoradiographic localization of vascular dopamine receptors, Am. J. Hypertens. 3, 34S.

Amenta, F., P. Gallo, A. Rossodivita and A. Ricci, 1993, Radioligand binding and autoradiographic analysis of dopamine receptors in the human heart, Arch. Pharmacol. 347, 147.

Aperia, A., B. Meister and T. Hukfelt, 1991, Dopamine: an intrarenal hormone, in contemporary issues, in: Nephrology, eds. S. Goldfard and F.N. Ziyadeh (Churchill Livingstone, Inc., New York) p. 315.

Bertorello, A. and A. Aperia, 1988, Pertussis toxic modulates dopamine inhibition of Na^+,K^+-ATPase activity in rat proximal convoluted tubule segments, in: The Na^+,K^+-pump, Part B: Cellular Aspects, eds. Skou J., J. Norby, A. Mausebafch and M. Esman (Liss, New York) p. 353.

Bertorello, A. and A. Aperia, 1990, Short-term regulation of Na^+,K^+-ATPase activity by dopamine, Am J. Hypertens. 3, 51S.

Bertorello, A.M., J.F. Hopfield, A. Aperia and P. Greengard, 1990, Inhibition by dopamine of (Na^+-K^+) ATPase activity in neostriatal neurons through D1 and D2 dopamine receptor synergism, Nature 347, 386.

Bevan, J.A., 1993, Flow regulation of vascular tone: its sensitivity to changes in sodium and calcium, Hypertension 22, 273.

Blaustein, M.P., 1977, Sodium ions, calcium ions, blood pressure regulation and hypertension: a reassessment and a hypothesis, Am. J. Physiol. 232, C165.

Brodde, O.E., 1990, Physiology and pharmacilogy of cardiovascular catecholamine receptors: implication for treatment of chronic heart failure, Am. Heart J. 120, 1565.

Burke, M.M., A.L. McGinn, D.C. Homans, B.V. Christensen, S.H. Kubo and R.F. Wilson 1995, Evidence for functional sympathetic reinnervation of left ventricle and coronary arteries after orthotopic cardiac transplantation in humans, Circulation 91 (1), 72.

Chen, C., R.E. Beach and M.F. Lokhandwala, 1993, Dopamine fails to inhibit renal tubular sodium pump in hypertensive rats, Hypertension 21, 364.

Creese, I. and D.R. Sibley, 1979, Radioligand binding studies: evidence for multiple dopamine receptors, Commun. Psychopharmacol. 3, 385.

De Weer, P., 1985, Cellular Sodium-Potassium transport. The Kidney: Physiology and Pathophysiology, ed. D.W. Seldin and G. Giebisch (Raven Press, New York) p. 35.

Felder, C.C., M. Blecher and P.A. Jose, 1989, Dopamine-1-mediated stimulation of phospholipase C activity in rat renal cortical membranes, J. Biol. Chem. 264, 8739.

Felder, C.C., P.A. Jose and J. Axelrod, 1989, The dopamine-1 agonist, SKF 82526, stimulates phospholipase-C activity independent of adenylate cyclase, J. Pharmacol. Exp. Ther. 248, 171.

Fleming, W.W., 1980, The electrogenic Na,K-pump in smooth muscle: physiologic and pharmacologic significance, Ann. Rev. Pharmacol. Toxicol. 20, 129.

Goldberg, L.I., 1975, The dopamine vascular receptor, Biochem. Pharmacol. 24, 651.

Gonzales, R.A. and F.T. Crews, 1985, Cholinergic-and-adrenergic-stimulated inositide hydrolysis in brain interaction, regional distribution, and coupling mechanisms, J. Neurochem. 45, 1076.

Hamlyn, J.K., M.P. Blaustein, S. Bova, D.W. Du Charme, D.W. Harris, F. Mandel, W.R. Mathews and J.K. Ludens, 1991, Identification and characterization of a ouabain-like compound from human plasma, Proc. Natl. Acad. Sci. USA 88, 6259.

Hernandez-R, J., 1992, Na^+/K^+-ATPase regulation by neurotransmitters, Neurochem. Int. 20, 1.

Hermsmeyer, K., 1976, Electrogenesis of increased norepinephrine sensitivity of arterial vascular muscle in hypertension, Circ. Res. 38, 362.

Jose, P.A., J.R. Raymond, M.D. Bates, A. Aperia, R.A. Felder and R.M. Carey, 1991, The renal dopamine receptors, J. Am. Soc. Nephrol. 2, 1265.

Kirkpatrick, K.A. and G. Burnstock, 1994, Release of endogenous ATP from the vasa deferentia of the rat and guinea-pig by the indirect sympathomimetic tyramine, J. Auton. Pharmacol. 14 (5), 325.

Lang, S., and M.P. Blaustein, 1980, The role of the sodium pump in the control of vascular tone in the rat, Circ. Res. 46, 463.

Mahan, L.C., R.M. Burch, F.J. Monsma and D.R. Sibley, 1990, Expression of striatal D_1 dopamine receptors coupled to inositol phosphate production and Ca^{2+} mobilization in Xenopus oocytes, Proc. Natl. Acad. Sci. USA, 87, 2196.

Middleton, J.P., J.R. Raymond, A.R. Whorton and V.W. Dennis, 1990, Short-term regulation of Na^+/K^+ adenosine triphosphatase by recombinant human serotonin $5\text{-}HT_{1A}$ receptor expressed in HeLa cells, J. Clin. Invest. 86, 1799.

Mulvany, M., 1985, Changes in sodium pump activity and vascular contraction, J. Hypertens. 3, 429.

Nishi, A., A.C. Eklof, A.M. Bertorello and A. Aperia, 1993, Dopamine regulation of renal Na, K-ATPase activity is lacking in dahl salt-sensitive rats, Hypertension 21, 767.

Patil, M.R., M.C. Satia, A.A. Mehta and R.K. Goyal, 1994, Evidence for catecholamine-depleting action of fluoxerine, Indian J. Physiol. Pharmacol. 38 (3), 169.

Rashed, S.M.K. and E. Songu-Mize, 1991, Effect of dopamine on sodium pump activity of the vascular smooth muscle; possible involvement of a pertussis toxin sensitive mechanism, Pharmacologist 33, 199 (Abstr. No. 310).

Rashed, S.M.K. and E. Songu-Mize, 1993a, Effect of dopamine on PI hydrolysis in rat vasculature, Pharmacologist 35, 142 (Abstr.).

Rashed, S.M.K. and E. Songu-Mize, 1993b, Signaling mechanisms involved in dopamine inhibition of vascular sodium, potassium adenosine triphosphatease, FASEB J. A815 (Abstr.).

Skou, J.C. and M. Esmann, 1992, The Na^+,K^+-ATPase, J. Bioenerg. Biomembr. 24, 249.

Smith, J.M., A.A. Sanchez and A.W. Jones, 1986, Comparison of rubidium-86 and potassium-42 fluxes in rat aorta, Blood Vessels 23, 297.

Songu-Mize, E., 1991, Vascular sodium pump activity kinetics in early and advanced stages of deoxycorticosterone-salt hypertension in rats, Life Sci. 49, 2045.

Songu-Mize, E., S.L. Bealer and R.W. Caldwell, 1987, Effect of DOCA salt treatment duration and anteroventral third ventricle lesions on a plasma-brone sodium pump inhibitor in rats, J. Hypertens. 5, 461.

Sun, M.K. and D.J. Reis, 1994, Dopamine or transmitter release from rat carotid body may not be essential to hypoxic chemoreception, Am. J. Physiol. 267, R1632.

Thomas, R.C., 1972, Electrogenic sodium pump in nerve and muscle cells, Physiol. Rev. 52, 563.

Vizi, E.S. and F. Oberfrank, 1992, Na^+/K^+-ATPase. Its endogenous ligands and neurotransmitter release, Neurochem. Int. 20, 11.

Yuasa, S., H. Bandai, T. Yura, T. Sumikura, N. Takahashi, K. Uchida, S. Miki, Y. Takamitsu and H. Matsuo, 1992, Dopaminergic modulation of the pressure-natriuresis response in rats, J. Hypertens. 10, 757.