

# Crosstalk between dopamine receptors and the Na<sup>+</sup>/K<sup>+</sup>-ATPase (Review)

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**Abstract.** Dopamine (DA) receptors, which belong to the G protein-coupled receptor family, are the target of ~50% of all modern medicinal drugs and constitute a large and diverse class of proteins whose primary function is to transduce extracellular stimuli into intracellular signals. Na<sup>+</sup>/K<sup>+</sup>-ATPase (NKA) is ubiquitous and crucial for the maintenance of intracellular ion homeostasis and excitability. Furthermore, it plays a critical role in diverse effects, including clinical cardioprotective and cardioprotective effects, ischemic preconditioning in the brain, natriuresis, lung edema clearance and other processes. NKA regulation is of physiological and pharmacological importance and has species- and tissue-specific variations. The activation of DA receptors regulates NKA expression/activity and trafficking in various tissues and cells, for example in the kidney, lung, intestine, brain, non-pigmented ciliary epithelium and the vascular bed. DA receptor-mediated regulation of NKA mediates a diverse range of cellular responses and includes endocytosis/exocytosis, phosphorylation/dephosphorylation of the  $\alpha$  subunit of NKA and multiple signaling pathways, including phosphatidylinositol (PI)-phospholipase C/protein kinase (PK) C, cAMP/PKA, PI3K, adaptor protein 2, tyrosine phosphatase and mitogen-activated protein kinase/extracellular signal-regulated protein kinase. Furthermore, in brain and HEK293T cells, D<sub>1</sub> and D<sub>2</sub> receptors exist in a complex with NKA. Among D<sub>1</sub> and D<sub>2</sub> receptors and NKA, regulations are reciprocal, which leads to crosstalk between DA receptors and NKA. In the present study, the current understanding of

signaling mechanisms responsible for the crosstalk between DA receptors and NKA, as well as with specific consequent functions, is reviewed.

## Contents

1. Introduction
2. Dopamine receptor-mediated regulation of NKA
3. NKA-mediated regulation of dopamine receptors or dopamine metabolism
4. Discussion and future prospects
5. Conclusion

## 1. Introduction

Over a number of decades, it has become evident that dopamine (DA) is important in the regulation of blood pressure and sodium balance by direct actions on renal, lung and intestinal epithelial, brain, non-pigmented ciliary epithelium and vascular bed ion transport, in which the regulation of Na<sup>+</sup>/K<sup>+</sup>-ATPase (NKA) exerts a critical role. There are five subtypes of DA receptors, D<sub>1</sub>, D<sub>2</sub>, D<sub>3</sub>, D<sub>4</sub> and D<sub>5</sub>. The D<sub>1</sub> and D<sub>5</sub> receptors are members of the D<sub>1</sub>-like family of DA receptors, whereas the D<sub>2</sub>, D<sub>3</sub> and D<sub>4</sub> receptors are members of the D<sub>2</sub>-like family. There is specific evidence indicating the existence of possible D<sub>6</sub> and D<sub>7</sub> receptors, however, such receptors have not yet been conclusively identified (1).

NKA, or the Na<sup>+</sup>/K<sup>+</sup> pump, is an energy transducing ion pump first described by Skou in 1957 (2), which uses ATP to transport Na<sup>+</sup> out of cells and K<sup>+</sup> into cells. The resulting gradient drives numerous processes, for example transport of glucose into intestinal and renal epithelial cells through a glucose-sodium cotransporter (3), as well as the transport of other nutrients, including amino acids (4) and ions, for example Ca<sup>2+</sup> (5). This enzyme is also responsible for generating the resting potential of cells, which is particularly important in neuronal and muscle function. It consists of an  $\alpha$  subunit (6), which is responsible for its catalytic and pharmacological properties, as well as  $\beta$

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and  $\gamma$  subunits, which may have regulatory functions (7,8). The  $\alpha$  subunit has four isoforms,  $\alpha_1$ ,  $\alpha_2$ ,  $\alpha_3$  and  $\alpha_4$ , while the  $\beta$  subunit has three isoforms,  $\beta_1$ ,  $\beta_2$  and  $\beta_3$ . The  $\gamma$  subunit is a hydrophobic protein of ~10 kDa. The  $\alpha_1$  isoform is expressed ubiquitously and plays the main 'housekeeping' role;  $\alpha_2$  is expressed primarily in skeletal muscle, as well as in the brain and heart;  $\alpha_3$  is expressed in the brain and heart; and  $\alpha_4$  is expressed in testis and skeletal muscle (9). It is reasonable to hypothesize that the tissue-specific distribution of the  $\alpha$  isoforms indicates that each isoform subtype exhibits a particular function associated with the tissue in which it is expressed. Animals lacking expression of the  $\alpha_1$  isoform die during embryogenesis; specifically, embryos fail to develop beyond the blastocyst stage (10). This is an expected result as the  $\alpha_1$  isoform is ubiquitously expressed and is required for multiple biological functions. Animals lacking the  $\alpha_2$  isoform gene are born but die immediately following birth (11). A subsequent study (12) demonstrated that  $\alpha_2$ -isoform knockout mice have a defect in the breathing center of the brain, causing failure to breathe and, thereby, death from asphyxia. The  $\alpha_1$  and  $\alpha_2$  isoforms regulate cardiovascular function. For example, studies indicate that the  $\alpha_2$  isoform of NKA mediates an ouabain-induced increase in vascular and cardiac contractility, which plays a role in the development and maintenance of hypertension (13) and cardiac inotropy in mice (14). Other studies have shown that the  $\alpha_1$  isoform may also regulate cardiac contractility and functionally and physically couple with the Na/Ca exchanger in the heart (15). Ouabain protects the heart against ischemia-reperfusion injury, which is likely due to activation of the NKA/c-Src receptor complex and subsequent stimulation of key mediators of preconditioning, such as phosphatidylinositol (PI)-phospholipase C (PLC)- $\gamma_1$  and protein kinase (PK) C- $\epsilon$  (16). The effect of NKA on increased cardiovascular risk, including ischemic heart failure, is likely to provide insight into the identification of new medicines. An example is the novel agent, istaroxime, which has inotropic (inhibition of NKA) and lusitropic (stimulation of sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase activity) effects in animal models of acute heart failure syndromes (17). Previous studies are consistent with the hypothesis that NKA activity significantly increases accompanied by an increased surface expression of the  $\alpha_1$  and  $\alpha_2$  isoforms of NKA, which protects neurons from subsequent ischemia 24-h following the preconditioning treatment (18). Therefore, NKA regulation becomes a crucial step in exploring subsequent responses in various tissues.

Multiple factors acting through signaling pathways, including protein kinases (cAMP/PKA, PKC and cGMP/PKG), Ca<sup>2+</sup>/calmodulin and reactive oxygen species, modulate the activity of NKA (19). DA receptor-mediated NKA regulation is of fundamental and clinical value and functions through the stimulation of multiple signaling pathways, including PLC/PKC, cAMP/PKA, PI3K, adaptor protein 2 (AP-2) and tyrosine phosphatase, resulting in diverse responses, for example the natriuretic effect, increased lung edema clearance, vascular tone, neuronal excitability and the neuroprotective effect. However, the DA receptor-NKA complex interacts and each part performs reciprocal functional modulation of the other (20). Therefore, this review presents a detailed discussion on the regulation of NKA by DA receptors via multiple pathways, followed by specific cellular responses and the modulation of DA receptors induced by NKA.

## 2. DA receptor-mediated regulation of NKA

It is well documented that DA increases or decreases the activity of NKA in an organ-specific manner. This regulation occurs, at least partially, via receptor-mediated second messenger activation or phosphorylation/dephosphorylation and promotes NKA insertion or removal from the plasma membrane. The activation of DA receptors induces opposing modulatory effects upon NKA in various tissue and cells, for example inhibition in the kidney and stimulation in alveolar epithelial cells (AECs). For example, the difference is due to phosphorylation of the NKA  $\alpha_1$  subunit in the kidney and dephosphorylation in the lung, leading to endocytosis/exocytosis, which in turn results in the decrease/increase of NKA activity and alternate second messenger activation. It is generally accepted that the signal pathways of NKA regulation by DA involve the stimulation of serine/threonine protein kinases, primarily PKA and PKC, as well as other pathways, including tyrosine kinase, AP-2, PI3K-PKC-G protein-coupled receptor kinase type 2 (GRK2) cascade and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathways.

*DA receptor-mediated inhibition of NKA in the kidney.* DA synthesized within the rat renal proximal tubule (PCT) is important for the regulation of renal sodium excretion, thus maintaining sodium homeostasis, a major determinant of blood pressure (21-23). The natriuretic effect of DA depends on its ability to increase the glomerular filtration rate and/or to directly modulate tubular sodium reabsorption by affecting NKA (24). It has previously been shown that phosphorylation of the NKA  $\alpha_1$  subunit Ser-11 or -18, in response to DA, provides the signal for NKA endocytosis. The inhibition of NKA activity in PCTs or a cell line derived from opossum proximal tubules (OK cells) (25,26) and the activation of PI3K, is critical for NKA endocytosis, as it favors binding of the AP-2  $\mu_2$  subunit to Tyr-537 within the  $\alpha_1$  subunit and, thereby, promotes clathrin recruitment (27-30).

Moreover, receptor-mediated internalization of NKA $\alpha$  and  $\beta$  subunits into early and late endosomes from the basolateral membrane (BLM) via a clathrin-dependent pathway and inhibition of NKA activity by DA in rat renal PCTs indicates a key role for activation of PI3K (27,31) in the endocytic sequence and demonstrates that this process is PKC dependent (32). In wild-type OK cells (33), early inhibition of NKA activity by DA is dependent on the stimulation of D<sub>1</sub> and D<sub>2</sub> receptors through activation of the PLC/PKC- and PLA<sub>2</sub>-dependent pathways, as well as late inhibition through PKA- and PLA<sub>2</sub>-dependent pathways. These pathways involve pertussis toxin (PTX)-sensitive G protein, resulting in the early downregulation of BLM expression of the  $\alpha$  subunit. By contrast, the total cell expression remains constant. Besides, the stimulation of D<sub>2</sub>-like receptors leads to the inhibition of NKA activity and hyperpolarization. These two effects are associated with the opening of K<sup>+</sup> channels and activation of PTX-sensitive G protein in OK cells (34). Furthermore, the DA-mediated regulation of NKA requires the PDZ-2 domain of sodium hydrogen regulatory factor-1 in OK cells (35). Ouabain, as an NKA inhibitor, was previously shown to decrease D<sub>1</sub> receptor-induced NKA inhibition in a human PCT line, resulting in increased renal

sodium reabsorption and eventual ouabain-induced hypertension (36). In human renal PCT cells, the scaffolding protein, caveolin-1, was revealed to be necessary for the association of D<sub>1</sub>-like receptors with G protein-coupled receptor kinase type 4 (GRK4) and the AP-2-associated reduction in plasma membrane NKA (37), among which the presence of gene variants at 3 exons in GRK4 is consistent with the known effects of GRK4 variants on uncoupling D<sub>1</sub> receptors from adenylate cyclase, demonstrating that GRK4 is a potential therapeutic target (38).

In PCT or OK cells, NKA inhibition via DA receptors is mediated by diverse signaling pathways in addition to phosphorylation of the NKA  $\alpha_1$  subunit. For example, DA produces a natriuresis, attributed, in part, to inhibition of NKA activity in PCT, and the impairment of this inhibition has been linked to several forms of hypertension in animals. These effects are largely mediated via D<sub>1</sub> or D<sub>1</sub>-like receptors (36) or by synergistic action of the D<sub>1</sub> and D<sub>2</sub> receptors (39) coupled to adenylyl cyclase activation and cAMP generation (40), as well as PLC/PKC signaling in rat renal PCT cells or Madin-Darby canine kidney epithelial cells (41,42). Dahl salt-sensitive rats lack the capacity to inhibit tubular NKA activity due to defective D<sub>1</sub> receptor adenylate cyclase coupling. This defect may contribute to the impaired natriuretic capacity in prehypertensive Dahl salt-sensitive rats (43). Those studies indicate that the failure of DA receptors to inhibit NKA activity contributes to animal models of hypertension (44), among which the adenylate cyclase pathway is important. Specific studies provided direct evidence that the D<sub>1</sub>-like receptor-mediated inhibition of NKA activity in OK cells involves a G protein of the G $\alpha_s$ , but not of the Gq/11 $\alpha$  class, positively coupled to adenylyl cyclase. Subsequent activation of PKA and PKC pathways follows in a single sequence of events with PKA activation prior to PKC activation, which likely includes phosphorylation of PLC by PKA (45). These observations suggest that the cAMP-PKA-PLC signaling cascade is important for the DA-induced PKC-mediated inhibition of renal NKA activity (45). Furthermore, among these signaling pathways, PKC is also important for DA receptor-induced NKA regulation. In OK cells, PKC-dependent inhibition of NKA activity by DA requires the integrity of the association between actin cytoskeleton and NKA (46). However, various PKC isoforms mediate with differing and even opposing effects. For example, PKC- $\beta$  and - $\zeta$  mediate opposing effects on PCT NKA activity. Phorbol 12-myristate 13-acetate-dependent stimulation of NKA is mediated by the activation of PKC- $\beta$ , whereas inhibition by DA requires the activation of PKC- $\zeta$  (47). Lipoic acid, while reducing oxidative stress, normalizes PKC activity and restores D<sub>1</sub> receptor-G(q/11) $\alpha$ -PLC signaling, along with the ability of SKF-38393 (D<sub>1</sub> receptor agonist) to inhibit NKA activity (41). These effects may infer value in clinical medication.

A previous study demonstrated that stimulation of the D<sub>5</sub> receptor decreases NKA activity in renal PCT cells from Wistar-Kyoto rats. Furthermore, pretreatment with a D<sub>3</sub> receptor agonist for 24 h enhanced D<sub>5</sub> receptor expression and the D<sub>5</sub> receptor-mediated inhibitory effect on NKA activity in Wistar-Kyoto cells, but decreased these effects in spontaneously hypertensive rat cells (48), indicating crosstalk of D<sub>3</sub> and D<sub>5</sub> receptors in NKA activity regulation. Wistar fatty rats tend

to develop salt-sensitive hypertension, which may be caused by excessive sodium retention occurring as a result of a defective dopaminergic system in the kidney that fails to inhibit NKA activity (49).

NKA is a transducer of signals between extracellular and intracellular compartments. Ouabain-stimulated NKA signaling (at ouabain concentrations far lower than those inhibiting pump activity) has recently demonstrated clinical promise by protecting malnourished embryonic kidneys from adverse developmental programming. A deeper understanding of the tissue-protective role of NKA signaling, as well as the regulation of NKA pumping activity, is of fundamental importance for the understanding and treatment of kidney diseases and kidney-associated hypertension (50).

*DA receptor-mediated stimulation of NKA in the lung.* By contrast, in AECs of normal rats, the stimulation of DA receptors increases lung edema clearance by increasing NKA function. Specific studies have shown that DA promotes protein phosphatase 2A translocation to the membrane fraction, leading to dephosphorylation of the NKA $\alpha_1$  subunit at the Ser-18 residue and its recruitment to the cell plasma membrane of alveolar epithelial type II (ATII) cells via DA receptors, which results in increased NKA activity (51,52) and lung fluid clearance (53). Moreover, new evidence has revealed that administration of a tyrosine-enriched diet to rats increases endogenous DA production, which causes increased NKA protein abundance in AECs, leading to increased NKA activity via the activation of D<sub>1</sub> receptors (54). Conceivably, the ability of AECs to produce DA may constitute a physiological mechanism favoring alveolar fluid reabsorption. In addition, the regulation of NKA by DA under pathological conditions exerts an important role. Studies have reported that rats exposed to 100% oxygen for 64 h demonstrate a decreased ability to clear edema in the isolated-perfused lung model, in association with decreased NKA activity in ATII cells (55). During acute hyperoxic lung injury, DA restores the ability of the lung to clear edema, which is mediated by recruitment and translocation of NKA from intracellular pools to the plasma membrane of the alveolar epithelium (56). This may be beneficial in the management of patients with acute hypoxemic respiratory failure. A previous study provided new evidence that DA exerts its effect by upregulating active Na<sup>+</sup> transport via the activation of amiloride-sensitive sodium channels and the basolateral NKA within minutes, which has been shown to be beneficial in a rat model of ventilator-induced lung injury (57). This information is relevant to current clinical trials exploring the effects of alveolar fluid clearance stimulation in patients with acute lung injury. Furthermore, overexpression of the NKA $\beta_1$  subunit led to increased alveolar fluid clearance in the rat model of acute hydrostatic pulmonary edema (58). Therefore, DA receptor-mediated stimulation of NKA in the lung provides a potential therapeutic option for the treatment of pulmonary edema.

DA-induced exocytosis of NKA is dependent on activation of PKC isozymes, PKC- $\epsilon$  and - $\delta$  (59). Additionally, DA enhances NKA activity in ATII cells by MAPK/ERK pathway proteins via the D<sub>2</sub> receptor by a mechanism involving *de novo* synthesis of  $\beta$  subunits and, possibly, recruitment of pre-existing  $\alpha$  subunits (60). Furthermore, DA activates

MAPK/ERK via Ras proteins, the serine/threonine kinase Raf-1 and diacylglycerol-dependent PKC isoenzymes through the D<sub>2</sub> receptor. However, importantly and contrary to the classical model, this pathway does not involve Grb2/SOS (adapter protein/guanine nucleotide exchange factor) complex formation in rat ATII cells (61). More studies are therefore required to elucidate the post-transcriptional regulatory mechanisms involved in DA-mediated NKA regulation.

*DA receptor-mediated regulation of NKA in the intestine.* The activity of intestinal NKA may also be affected or modulated by DA. The inhibition of jejunal NKA activity through D<sub>1</sub> receptors is mediated by locally formed DA and is dependent on salt intake in 20-day-old rats (62) and obese rats (63). By contrast, in lean Zucker rats, NKA fails to respond to the activation of D<sub>1</sub> receptors irrespective of their salt intake (63). Furthermore, the association between salt intake, increased DA formation and inhibition of NKA at the intestinal level is not as straightforward as that described in renal tissues. For example, in old-aged rats, a high salt diet fails to alter the intestinal dopaminergic tonus or NKA activity, whereas in adult rats this diet decreases NKA activity (64). DA inhibits jejunal NKA in young breast-fed Wistar rats through the activation of D<sub>1</sub> receptors, but not in adult rats or in young rats fed solid food for two days. The lack of DA sensitivity is accompanied by markedly elevated basal jejunal NKA activity (65). Therefore, food intake in young rats is important in the development of the DA insensitivity by NKA activity. These studies indicate that DA receptor-mediated regulation of NKA in the intestine remains controversial.

Additionally, jejunal NKA activity in young rats is regulated by cholera toxin (CTX)- and PTX-sensitive G proteins, however, regulation of NKA by CTX-sensitive G proteins is absent in adult animals. This difference may explain the failure of DA to inhibit intestinal NKA activity in adult rats (66). Furthermore, the uninephrectomised and three-quarter nephrectomised rats (3/4nx rats, a model of chronic renal insufficiency) present DA-sensitive enhanced natriuresis. In uninephrectomised rats, this is accompanied by a reduced jejunal NKA activity (67), whereas in 3/4nx rats, by an increased jejunal NKA activity (68). These studies indicate that the changes in jejunal NKA activity may contribute to the maintenance of sodium homeostasis in conditions of compromised renal function and that there are complementary functions between the intestine and kidney during development and renal-intestinal crosstalk.

*DA receptor-mediated inhibition of NKA in the brain, non-pigmented ciliary epithelium and vascular bed.* The modulation of NKA by DA also occurs in other tissues, for example, the synergism between D<sub>1</sub> and D<sub>2</sub> receptors underlies numerous electrophysical and behavioural effects of DA in the mammalian brain. Previous studies have provided evidence for joint and separated confinement of the D<sub>1</sub> receptor and NKA in the postsynaptic areas of dendritic spines (69). DA inhibits the NKA activity of isolated striatal neurons through a synergistic effect on D<sub>1</sub> and D<sub>2</sub> receptors (70,71), while fencamfamine, acting as an indirect DA agonist, reduces NKA activity through cAMP-dependent PKA mechanisms via D<sub>1</sub> and D<sub>2</sub> receptors in rat striatum (71). These studies support

the hypothesis that DA and other neurotransmitters regulate neuronal excitability through the novel mechanism of pump inhibition. Besides, in rat brain, inhibition of NKA activity and mitochondrial respiratory chain function (various toxic actions) by DA may trigger intracellular damage pathways leading to the mortality of nigral dopaminergic neurons (72). D<sub>1</sub>- and D<sub>2</sub>-like receptor activation contribute to neuronal death in the striatum of newborn piglets after hypoxic-ischemic encephalopathy in association with increased nitrotyrosine and decreased NKA activity. Additionally, the mechanisms of D<sub>1</sub>-like receptor toxicity may involve cAMP-regulated phosphoprotein of 32 kDa-dependent phosphorylation of *N*-methyl-D-aspartate (NMDA) receptor NR<sub>1</sub> and NKA (73). However, the maintenance of adequate cerebral perfusion pressure, by pharmacologically preventing systemic hypotension with DA infusion, has been found to prevent cerebral ischemia and attenuates energy depletion and neuronal injury in a model of newborn piglets with meningitis. In this model, the decreased cerebral cortical cell membrane NKA activity and increased lipid peroxidation products, indicative of meningitis-induced brain damage, are significantly attenuated by DA infusion (74). Similarly, the decreased DA levels in the brain may be partly responsible for the decrease in NKA activity in the striatum of newborn piglets during posthypoxic reoxygenation (75). A further study revealed that D<sub>2</sub> receptors are involved in stimulating striatal NKA activity following short-term morphine treatment, whereas D<sub>1</sub> receptors are involved in inhibiting striatal NKA activity subsequent to long-term morphine treatment (76). Therefore, bidirectional regulation of NKA by DA in the brain indicates its complexity and implications in neuroprotective therapy (Fig. 1).

In rabbit non-pigmented ciliary epithelium, stimulation of the D<sub>1</sub> receptor causes a reduction of NKA-mediated ion transport and NKA activity by a mechanism that may involve a tyrosine kinase step and the cAMP/PKA pathway (77).

Moreover, DA inhibits rat tail artery NKA activity, which the D<sub>1</sub> and D<sub>2</sub> receptor subtypes mediate. It appears that D<sub>1</sub>, but not D<sub>2</sub> receptor agonist-induced inhibition of NKA is mediated by a PTX-sensitive mechanism and may be coupled to the activation of the PLC system. Modulation of NKA by DA may contribute to vascular tone (78). Moreover, DA and DA receptor agonists inhibit NKA activity through D<sub>1</sub> receptors, which are linked to PTX sensitive-mechanisms and the PLC signaling pathway in cultured rat aortic smooth muscle cells (79) (Fig. 1).

### 3. NKA-mediated regulation of DA receptors or DA metabolism

It has been well documented that DA increases or decreases the activity of NKA in an organ-specific manner via receptor-mediated second messenger activation and promotes NKA insertion or removal from the plasma membrane. However, the first evidence of a direct interaction between NKA and DA receptors has also been reported. Furthermore, NKA and DA receptors are able to functionally regulate one another via protein-protein interactions in the absence of ligands or downstream signaling events, indicating that, in addition to traditional second messenger-mediated communication, NKA and DA receptors associate in a complex to provide a more

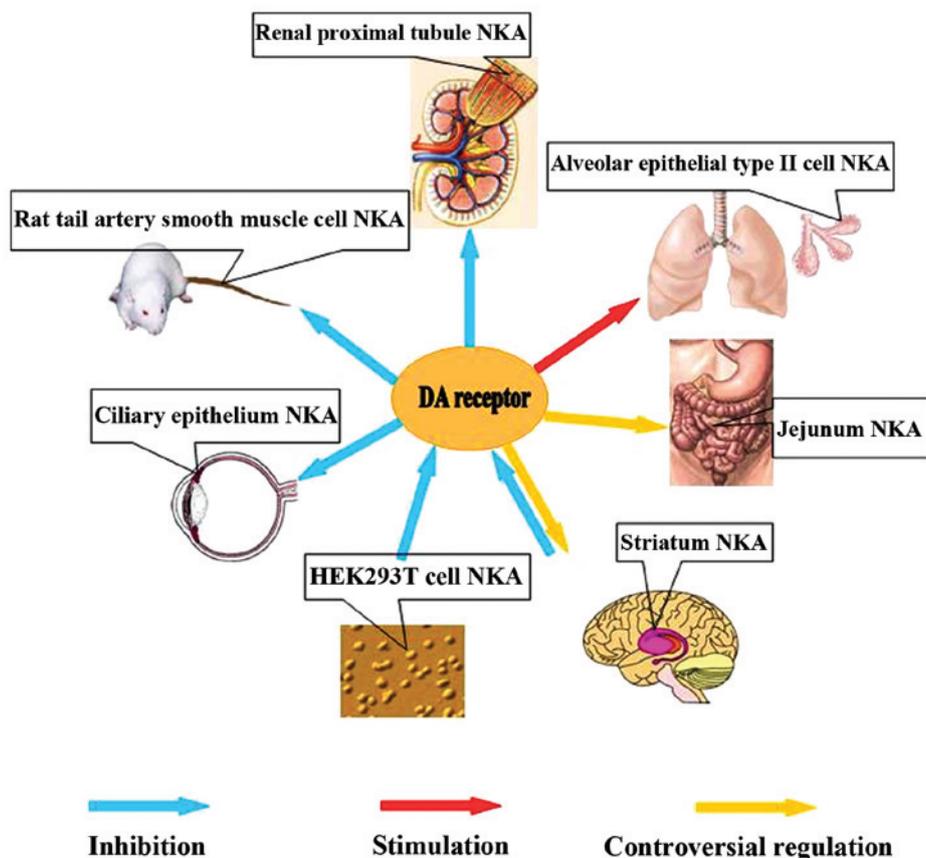


Figure 1. Dopamine receptor-mediated regulation of NKA in the kidney, lung, intestine, brain, non-pigmented ciliary epithelium and vascular bed. DA, dopamine; NKA,  $\text{Na}^+/\text{K}^+$ -ATPase.

rapid and immediate response to external stimuli or changes to the cellular environment. Additionally, NKA association with the  $\text{D}_1$  and  $\text{D}_2$  receptors leads to decreased signaling through a novel mechanism that is independent of receptor density or trafficking. This novel finding of DA receptor and NKA interaction was confirmed by co-immunoprecipitation and western blot analyses in HEK293T cells and this was further substantiated by verifying the interaction between native DA receptors and NKA in brain tissue (20). Moreover, an increased intracellular sodium concentration induces the increased colocalization of DA receptors with NKA molecules in the region of the plasma membrane of renal epithelial cells (80). Ouabain, as a NKA inhibitor, decreased  $\text{D}_1$  receptor-induced NKA inhibition in the human PCT cell line, which was responsible for the increase in renal sodium reabsorption and eventually led to ouabain-induced hypertension (36). This indicates that NKA inhibition is important in the regulation of DA receptor-induced sodium homeostasis and the subsequent regulation of blood pressure. Cannabinoids and arachidonic acid have been found to inhibit DA and 5-HT uptake into rat neocortical synaptosomes; this effect was neither cannabinoid receptor-mediated nor due to competitive inhibition of membrane transporters, but was partly affected by a decreased NKA activity (81). Furthermore, in HEK293T cells with NKA and DA receptor co-expression, the expression of NKA was found to markedly decrease  $\text{D}_1$  and  $\text{D}_2$  receptor densities with a concomitant functional decrease in DA receptor-mediated regulation of cAMP levels. However,

pharmacological inhibition of endogenous or overexpressed NKA enhance DA receptor function without altering receptor number or localization. Similarly, DA receptor function was also enhanced by small-interfering RNA reduction of endogenous NKA. These observations indicate that, under basal conditions, NKA negatively regulates DA receptor function via protein-protein interactions. Expression of DA receptors decreases endogenous NKA function in a reciprocal manner in the absence of DA, suggesting DA receptor proteins as regulators of NKA activity (20). This crosstalk of reciprocal regulation between DA receptors and NKA provides a novel control mechanism for DA receptor signaling and cellular ion balance.

#### 4. Discussion and future prospects

*DA receptor-mediated regulation of NKA by synergism with other substances.* The synergism of DA and 20-hydroxyeicosatetraenoic acid, a major arachidonic acid metabolite of cytochrome P450, inhibits NKA activity in PCT via the  $\text{D}_1$  signaling pathway (82). Urodilatin or DA decreases NKA activity, while urodilatin and DA combined, further decrease NKA activity, demonstrating an additive effect on the sodium pump, suggesting that urodilatin and DA act via a common intracellular pathway to decrease sodium and water tubular reabsorption, contributing to its natriuretic and diuretic effects (83). Additionally, synergistically interacting  $\text{D}_1$  and NMDA receptors mediate non-vesicular transporter-dependen-

dent  $\gamma$ -aminobutyric acid (GABA) release in rat striatal medium spiny neurons through cAMP-dependent inhibition of NKA. This induces the accumulation of intracellular sodium, reversal of the GABA carrier and potentiation of NMDA-induced release (84). Stimulation of the D<sub>3</sub> receptor inhibited NKA activity, which was increased by pretreatment with the endothelin B-receptor agonist, BQ3020, in renal PCT cells of Wistar-Kyoto rats, but not in renal PCT cells of spontaneously hypertensive rats (85). By contrast, pretreatment with PD128907, a D<sub>3</sub> receptor agonist, elevated the inhibitory effect of BQ3020 on NKA activity in Wistar-Kyoto, but not in spontaneously hypertensive cells (86). Furthermore, insulin increases D<sub>5</sub> receptor-mediated NKA inhibition and the aberrant interaction between insulin and D<sub>5</sub> receptors regulates renal sodium transport and participates in the pathogenesis of hypertension (87). These synergistic effects of DA receptors and other substances play a crucial role in the regulation of NKA and associated clinical diseases.

*DA receptor-mediated regulation of NKA by antagonism with other substances.* The selective activation of 5-HT<sub>1A</sub> receptors increases NKA activity in renal cortical tubules, responsible for the antinatriuretic effect of 5-HT and antagonizes NKA inhibition by DA (88). Similarly, in the intestine, the inhibitory action of maximal doses of DA on NKA activity is significantly prevented by coinubation with 5-HT, indicating the presence of a functional antagonism between the two amines in the control of NKA activity (62).

Additionally, angiotensin receptor subtypes (AT<sub>1</sub>) and D<sub>1</sub> receptors function as a unit of opposites, which may provide a highly versatile and sensitive system for short-term regulation of sodium excretion (89). The stimulation of renal AT<sub>1</sub> receptors by angiotensin II (Ang II) delivers signals through the PLC pathway to inhibit extraneuronal DA uptake. Ang II was able to stimulate renal NKA activity alone, while DA and Ang II functioned together through a common pathway involving reversible renal tubular NKA deactivation and activation, respectively (90). Ang II induces a rapid partial internalization of D<sub>1</sub> receptors and elimination of D<sub>1</sub> receptor signaling in rat renal PCT cells, resulting in a change in NKA activity. Similarly, exposure to a D<sub>1</sub> agonist results in rapid partial internalization of AT<sub>1</sub> receptors and complete elimination of AT<sub>1</sub> receptor signaling. D<sub>1</sub> and AT<sub>1</sub> receptors are partners in a multiprotein complex. NKA, the target for the two receptors, is included in this complex and a region in the COOH-terminal tail of D<sub>1</sub> receptors (residues 397-416) is found to interact with AT<sub>1</sub> receptors and NKA (89). These studies indicate that AT<sub>1</sub> and D<sub>1</sub> receptors function as a unit of opposites, which provides a highly versatile and sensitive system for short-term regulation of sodium excretion.

Additionally, *in vivo* short-term morphine treatment has been found to stimulate NKA activity by inhibiting PKA activity and subsequently decreasing NKA phosphorylation in a dose-dependent manner, which may be significantly inhibited by the D<sub>2</sub>-like receptor antagonist, eticlopride. Contrary to short-term morphine treatment, long-term morphine treatment significantly suppressed NKA activity by stimulating PKA activity and subsequently increasing the NKA phosphorylation, which may be significantly inhibited by the D<sub>1</sub>-like receptor antagonist, SCH 23390 (76). These observations demonstrate

that DA receptors are involved in the regulation of NKA activity following activation of opioid receptors by morphine (76).

Similarly, in rat renal PCT cells, NKA activity is stimulated by Ang II and oxymetazoline (an  $\alpha$ -adrenergic agonist) at physiological, nonsaturating Na<sup>+</sup> concentrations, while these stimulatory effects are blocked by DA and atrial natriuretic peptide, as well as by their respective second messengers, cAMP and cGMP (91). DA receptor-induced NKA regulation is antagonized by other hormones, including insulin. Insulin activates the PI3K-PKC-GRK2 cascade, causing D<sub>1</sub> receptor serine phosphorylation, which leads to D<sub>1</sub> receptor down-regulation and uncoupling from G proteins. This results in the failure of D<sub>1</sub> receptor agonists to stimulate G proteins and inhibit NKA activity (92,93).

Furthermore, DA oxidation products, for example H<sub>2</sub>O<sub>2</sub> and reactive quinones, have been responsible for various toxic actions of DA in rat brain, including the inhibition of NKA activity and mitochondrial respiratory chain function, which may trigger intracellular damage pathways leading to the death of the nigral dopaminergic neuron. The H<sub>2</sub>O<sub>2</sub>- and quinone-scavenging properties of *N*-acetylcysteine are likely to account for its protective effect against NKA inhibition induced by DA. The results have important implications in the neuroprotective therapy of sporadic Parkinson's disease (72). Therefore, the regulation of NKA by DA may be important for the development of neuroprotective therapy.

The bidirectionally regulated activity of NKA indicates the complexity of NKA regulation in sodium reabsorption *in vivo*. These may infer certain value in clinical medication.

*DA receptor-mediated regulation of NKA by affecting mutual release or turnover, as well as catabolism between DA and other substances.* DA modulates NKA by affecting the mutual release or turnover and catabolism between DA and other hormones. For example, D<sub>2</sub> receptors have been implicated in stimulating striatal NKA activity following short-term morphine treatment, whereas D<sub>1</sub> receptors are involved in inhibiting striatal NKA activity subsequent to long-term morphine treatment. In this case, *in vivo* short-term injection of morphine is able to promote DA release and activate D<sub>2</sub> receptors, resulting in NKA stimulation, whereas *in vitro* direct administration of morphine to isolated striatal synaptosomes is not the result of non-functional striatal circuits or inhibition of the afferent input signal (76).

Atrial natriuretic factor (ANF) may decrease renal DA turnover and catabolism, favor DA accumulation into renal cells and increase its endogenous content and availability. This permits D<sub>1</sub> receptor recruitment and stimulation and, in turn, inhibition of NKA activity resulting in decreased sodium reabsorption. ANF and DA may act in this way via a common pathway to enhance natriuresis and diuresis (94). Therefore, increased effort into the study of NKA regulation *in vivo* is essential and is also critical for clinically relevant medication in hormone synergism and counteraction of NKA regulation, which results in differing responses.

## 5. Conclusion

DA-mediated regulation of NKA through multiple signal transduction pathways (cAMP/PKA, PI-PLC/PKC, PI3K,

AP-2, tyrosine phosphatase and MAPK/ERK) results in diverse responses, including the natriuretic effect, lung edema clearance, vascular tone, neuronal excitability and neuroprotective effects. These results suggest that NKA regulation by DA is affected by complex functional networks that are either specific or non-specific and involve distinct and often mutually interacting intracellular signal transduction pathways. Conversely, NKA also regulates DA receptors, indicating the complicated crosstalk between DA receptors and NKA. Accordingly, more experiments should be conducted on the complexity of various substances interacting with NKA activity *in vivo*, as these may be of value in clinically relevant medication. In conclusion, understanding the crosstalk of DA receptors and NKA, as well as further investigation into this function, are important for the interest of drug development.

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