Dopaminergic Modulation of Na,K-ATPase Activity in the Proximal Tubules of Normotensive and Hypertensive Rats

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Renal proximal tubular Na,K-ATPase plays an important role in the maintenance of sodium homeostasis and it is known that dopamine (DA) exerts an inhibitory effect on the activity of this enzyme. We have found that DA-induced inhibition of Na,K-ATPase is abolished in the spontaneously hypertensive rats (SHR) in comparison with age-matched Wistar-Kyoto (WKY) rats. Dopamine inhibits Na,K-ATPase via phospholipase C coupled protein kinase C pathway. The enzyme protein kinase C subsequently causes inhibition of Na,K-ATPase. In the SHR, DA-induced activation of phospholipase C is diminished, which in turn is responsible for the abolished inhibition of Na,K-ATPase. We have now shown that DA-induced activation of protein kinase C, which results from activation of DA-1 receptors is also abolished in the SHR which would account for the failure of DA to inhibit Na,K-ATPase in the hypertensive animals. Recently, we have examined the possibility that the failure of DA to inhibit Na,K-ATPase activity may be related to abnormal expression of DA receptors. In radioligand binding studies with [3H] SCH 23390 as a DA-1 receptor ligand and [3H] spiroperidol as a DA-2 receptor ligand we showed that both [3H] SCH 23390 and [3H] spiroperidol bindings are best fit to one site model in either WKY or SHR. Both B\text{max} and K\text{D} of either ligand binding to proximal tubule in the SHR were not statistically different from their WKY counterparts. Therefore, these results show that failure of DA to inhibit Na, K-ATPase activity in the SHR is most likely due to a defective coupling of DA-1 receptor to its G protein on the basolateral membrane, but not due to abnormal expression of DA receptors. (Hypertens Res 1995; 18 Suppl. I: S43-S46)

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Dopamine (DA) and selective DA receptor agonists produce changes in cardiovascular and renal function which include hypotension resulting from a decrease in peripheral vascular resistance, and an inhibition of renal tubular sodium reabsorption resulting in natriuresis and diuresis (1). Recent studies from our and other laboratories have established that it is the activation of DA-1 subtype of DA receptors which is responsible for the natriuretic response observed during infusion of DA receptor agonists (2-4). In addition, it has also been reported that the natriuretic response to either exogenously administered DA or endogenously produced DA is diminished or attenuated in the spontaneously hypertensive rats SHR in comparison with their normotensive counterpart, Wistar-Kyoto (WKY) rats (5-7). This review will discuss the cellular signaling mechanisms involved in DA-1 receptor mediated natriuresis and diuresis and attempt to identify the abnormality in these putative mechanism(s) which may be responsible for the diminished response to DA receptor agonists in the SHR.

Dopamine Receptor Mediated Cellular Signaling Mechanisms in the Proximal Tubule

It is shown that DA receptors of DA-1 and DA-2 subtypes are localized both on the brushborder and basolateral membranes of the proximal tubule (8). However, in most published studies it is the cellular signaling mechanisms activated as a result of DA-1 receptor stimulation, which appears to be responsible for DA-induced inhibition of sodium reabsorption and subsequent natriuresis (9-12). Studies conducted on DA-1 receptor coupled signal transduction pathways on the brushborder membrane have revealed that stimulation of cyclic AMP is responsible for the inhibition of Na/H antiport produced by DA and selective DA-1 receptor agonists (13). Since the mechanisms leading to this response at the brushborder membrane have been investigated in detail by other investigators (13,14), the present review will focus on DA-1 receptor-mediated cellular signaling mechanisms on the basolateral side of the proximal tubule.

In our laboratories, we have carried out a systematic study to identify DA-1 receptor mediated
activation of different enzymes on the basolateral membrane, which ultimately results in the inhibition of the Na,K-ATPase by dopamine. Our initial studies showed that DA causes activation of the enzyme phospholipase C which involved activation of DA-1 receptors as well as alpha-adrenoceptors (15). This finding is consistent with the receptor pharmacology of DA in that at lower doses DA activates specific DA receptors and alpha and beta-adrenoceptor activation is seen at higher doses of DA (16). The physiological significance of DA-induced activation of phospholipase C was further examined in a study in which we showed that in rats placed on high sodium intake the increase in urinary sodium excretion was accompanied by a parallel increase in urinary DA excretion as well as an increase in the activity of renal cortical phospholipase C (17). It is likely that the increased phospholipase C activity accounted for the greater inhibition of Na,K-ATPase reported in rats placed on high sodium intake (18) and subsequent natriuresis and maintenance of sodium balance in these animals.

It is known that diacylglycerol and inositol triphosphate are generated following the activation of phospholipase C, and diacylglycerol activates the enzyme protein kinase C (19). We conducted studies to directly determine the effect of DA on protein kinase C activity in the renal proximal tubules and our results show that DA activates protein kinase C by stimulating DA-1 receptors (unpublished observations). This was based upon the findings that the stimulatory effect of DA on protein kinase C activity was mimicked by fenoldopam, a DA-1 receptor agonist, but not by bromocriptine, a DA-2 receptor agonist. Furthermore, the effects of DA and fenoldopam were antagonized by the DA-1 receptor antagonist SCH 23390. Similarly, we have also found that DA causes inhibition of Na,K-ATPase by activating DA-1 receptors since this effect was mimicked by a selective DA-1 receptor agonist but not by bromocriptine, a DA-2 receptor agonist. The fact that it was a defect in DA-ergic signal transduction pathway was proven when it was discovered that direct activation of protein kinase C by either OAG or PDBu led to inhibition of Na,K-ATPase activity in the SHR. The degree of inhibition of the enzyme activity in SHR was similar to that seen in the WKY rats (21).

In order to examine the mechanisms responsible for the diminished response to DA receptor agonists in the SHR, we undertook a series of studies designed to determine the effects of DA on DA-1 receptor coupled cellular signaling mechanisms in the basolateral membrane of the SHR.

We discovered that DA failed to inhibit Na,K-ATPase by activating DA-1 receptors since this effect was mimicked by a selective DA-1 receptor agonist but not by DA-2 receptor agonist and it was blocked by a selective DA-1 receptor antagonist but not by DA-2 receptor antagonist (20). We have utilized inhibitors and activators of the enzymes affected by DA and found that DA inhibits the basolateral Na,K-ATPase by a phospholipase C coupled protein kinase C pathway (21). This conclusion is based upon our findings that the compound U-73122, an inhibitor of phospholipase C, prevents the inhibitory effect of DA on Na,K-ATPase activity and that a diacylglycerol analog, 1-oleoyl-2-acetyl-racglycerol (OAG), and a direct activator of protein kinase C, phorbol 12,13-dibutyrate (PDBu), mimic the inhibitory effect of DA on Na,K-ATPase (21). A diagrammatic representation of the DA-1 receptor mediated signaling pathway on the basolateral membrane of the proximal tubule is shown in Fig. 1.

**Dopaminergic Modulation Cellular Signaling Mechanisms in the Proximal Tubules of Spontaneously Hypertensive Rats**

In order to examine the mechanisms responsible for
no differences in DA-1 receptor numbers and affinity between SHR and WKY rats suggesting that DA receptor expression is not altered in the SHR. Therefore, it appears that the most likely explanation for the inability of DA to activate the cellular signaling pathway in the SHR as effectively as in WKY rats results from the uncoupling of DA-1 receptors from the G protein on the basolateral membrane. Such an explanation is consistent with a similar defect in DA-1 receptor/adenyl cyclase coupling on the brushborder membrane in the SHR (23,24) and would account for the diminished natriuretic response to DA and DA-1 receptor agonists in spontaneously hypertensive rats.

References


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**Fig. 2.** The effect of dopamine on protein kinase C (PKC) activities in renal proximal tubules obtained from the normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR). N = 6 animals per group; two animals were used for the preparation of proximal tubular suspensions for each experiment; incubations were performed in triplicate. Data are presented as MEAN±SEM. * Significantly different compared with the corresponding control within the same group; ** significant difference between SHR and WKY rats.

**Fig. 3.** Specific binding of [3H] SCH 23390 to proximal tubules of spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats, as a function of ligand concentration. Non-specific binding was determined in the presence of 10 μM SCH 23390. The figure shows plots representative of four individual experiments, each performed in duplicate in tubules obtained from SHR and WKY rats. Insets within each plot are the scatchard analysis of the binding data and show no apparent difference in either B_max or K_D between SHR and WKY rats.
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