

# Dopamine in the developing kidney

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**ABSTRACT** The adult kidney has a high rate of dopamine (DA) production, metabolism, and signalling. The non-neuronal DA system in the adult kidney is of utmost importance for the regulation of salt metabolism. DA may also act as a transcription factor and may be of importance for tissue differentiation. In the central nervous system, D1 receptors require the dopamine- and cAMP-regulated phosphoprotein with a molecular weight of 32,000 Dalton (DARPP-32) to mediate their actions. The renal D1 mediates DARPP-32 activation via a cascade involving cAMP and PKA, and protein kinase C (PKC) activation via phospholipase C. Active DARPP-32 has a specific inhibitory effect on protein phosphatase 1 (PP1), leaving, e.g. Na<sup>+</sup>, K<sup>+</sup>-ATPase in a phosphorylated, inactive, state. Thus, dopamine acts as a natriuretic hormone in the mature kidney. Here, we discuss the age-dependent distribution and some functional aspects of several parts of the renal dopamine system (dopamine, AADC, COMT, D1 receptor, and DARPP-32) during renal morphogenesis.

**KEY WORDS:** *dopamine, DARPP-32, kidney morphogenesis*

## Background

The non-neuronal dopamine system in the mature kidney is of utmost importance for the regulation of salt metabolism. Intrarenally formed dopamine acts as a natriuretic hormone by inhibiting Na<sup>+</sup>,K<sup>+</sup>-ATPase and other sodium transporters in several tubular segments (for reviews Aperia, 1994, 1998).

In the mature kidney the dopamine precursor, L-deoxyphenylalanine (L-DOPA), present in the primary urine, is taken up by proximal tubular cells, which have a high concentration of L-aromatic amino acid decarboxylase (AADC). AADC converts L-DOPA to dopamine. This locally formed dopamine acts as an autocrine and paracrine factor in the nephron and can be metabolised by catechol-O-methyl transferase (COMT) and monoamine oxidase (MAO) in several tubular segments. AADC, COMT and MAO are all abundantly expressed in tubular cells of the mature kidney.

There are principally two types of dopamine receptors, which from the beginning were characterised with regard to their capacity to activate (D1) or inhibit (D2) the activity of adenylyl cyclase (Missale *et al.*, 1998). Both subtypes of dopamine receptors have been identified in the kidney. The D1 subtype of dopamine receptors is expressed in the mature kidney. The renal D1 subtype of receptors has also been shown to couple to the phospholipase C-PKC signalling pathway. In the central nervous system, D1 receptors require the dopamine- and cAMP-regulated phosphoprotein with a molecular weight of 32,000 Dalton (DARPP-32) to mediate their actions. Depend-

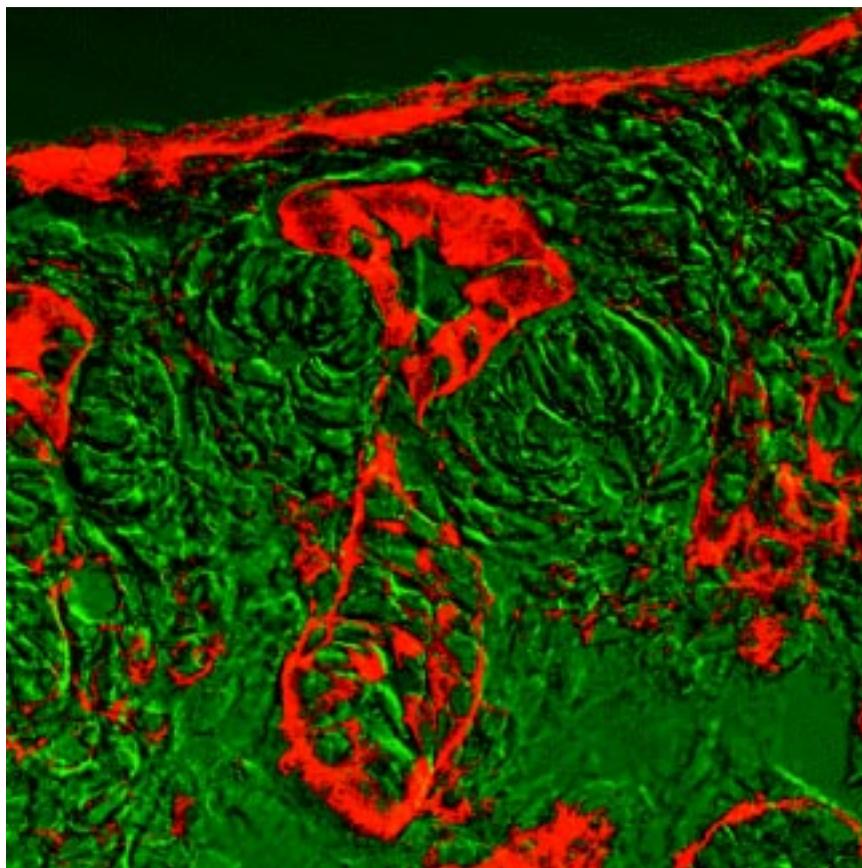
ing on the activity of protein kinases, such as PKA, and protein phosphatases, such as calcineurin, DARPP-32 cycles between an active, phosphorylated, and an inactive, dephosphorylated, state. Active DARPP-32 has a specific inhibitory effect on protein phosphatase 1 (PP1). Hence, DARPP-32 will funnel D1 and D2 dopaminergic signals towards its target protein, PP1. In the mature kidney, phosphorylation and activation of DARPP-32 leads to inhibition of the activity of Na<sup>+</sup>,K<sup>+</sup>-ATPase.

## Dopamine during renal development - recent progress

There is a great deal of evidence suggesting that dopamine may also act as a transcription factor and may be of importance for tissue differentiation. Hence, it is important to elucidate the localisation and activity of the dopamine system in the developing kidney. We have studied the expression of dopamine, its synthesising and metabolising enzymes, the D1 subtype of receptor and some of the signalling systems activated by dopamine in the embryonic and postnatal rat kidney. Using confocal microscopy, we have, by immunofluorescent technique, examined the age-dependent distribution of the various proteins related to the dopamine system in the developing rat kidney. We have also performed some *in situ* hybridisation studies.

*Abbreviations used in this paper:* E, embryonic day; PN, postnatal day.

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**Fig. 1. Immunofluorescence for DARPP-32 (red signal, superimposed on green DIC micrograph) in an E21 rat kidney.** Note the expression of DARPP-32 in the tip of the ureteric bud. There is no signal in the mesenchyme or condensates on either side of the ureteric bud.

AADC protein is detected in the periphery of E15 to E21 rat kidneys. At PN5, AADC is located to newly formed proximal tubules. Dopamine-like immunoreactivity is present in E21 tubules, mainly in the basolateral region of the cell. It is absent in glomeruli. COMT protein appears late in nephrogenesis. It is first detected at E18 in primitive proximal tubules and collecting ducts. It is abundant in tubules of postnatal and adult rat kidneys.

The D1 subtype of receptor is present in all cells from E15 throughout nephrogenesis and into adulthood. The relative amount of the D1 receptor appears to increase with age. DARPP-32 is not detectable until E18. At E18, DARPP-32 mRNA and protein appears at the tip of the ureter in the nephrogenic zone (Fig. 1). The localisation of DARPP-32 is interesting, since it mimics that of tyrosine kinase receptors *c-ros* and *c-ret*, which are of critical importance for normal renal development (Pichel *et al.*, 1996). However, in contrast to *c-ret*, DARPP-32 is absent in earlier developmental stages. The expression of DARPP-32 in the ureter tips persists from E18 till the end of nephrogenesis. In the postnatal kidney, DARPP-32 is present in the inner medulla. Inhibitor-1, another PP1 inhibitor, which is structurally similar to – but differently regulated than – DARPP-32, is expressed specifically in the peripheral stem cells of E15 rat kidneys (Svehnilson *et al.*, 1995), but disappears in late nephrogenesis (J. Svehnilson *et al.*, unpublished observation). The expression

of Inhibitor-1 in adult rat kidney is similar to that of DARPP-32.

Transgenic mice lacking functional DARPP-32 (Fienberg *et al.*, 1998), display normal anatomy and histology of the central nervous system, but suffer from severe neurological and psychological disturbances. We have, in an ongoing study, found that DARPP-32 deficient mice have significantly fewer nephrons and a significant reduction in kidney weight/body weight ratio as compared with their wild-type controls. Apart from the smaller kidney size and the reduced number of nephrons, the histology of DARPP-32 knockout kidneys appears unremarkable and there are no signs of compensatory hypertrophy.

The ser/thr protein phosphatases, PP1 and PP2A, are both ubiquitously expressed in E15 rat kidney. The activity of PP1, located downstream from DARPP-32 in the dopamine - D1-cAMP-DARPP-32 pathway (see above), is required during nephrogenesis. In E13 rat metanephric explants inhibition of PP1 and PP2A with okadaic acid leads to impaired morphogenesis and increased apoptosis (Svehnilson *et al.*, 1995). In the mature kidney, dopamine may also use PKC as a signalling molecule. In metanephric organ culture inhibition of PKC with Ro 31-8220 or the endogenous signal transducer ceramide results in dysmorphogenesis and apoptosis (Serlachius *et al.*, 1997).

## Conclusion

In summary, these results suggest that the dopamine system is an important modulator of renal differentiation and growth in late nephrogenesis. The specific localisation of the dopamine signal effector DARPP-32 to the ureter tips and the observation that DARPP-32 deficient mice lack a significant number of nephrons as compared with wild-type littermates suggest that DARPP-32 is an important signal transducer during nephrogenesis. Since DARPP-32 appears only during the later part of kidney embryogenesis, we speculate that this phosphoprotein is of particular importance for the formation of superficial nephrons. Since superficial and juxtamedullary types of nephrons have distinct properties with regard to salt and water handling, disturbances in the development of the renal dopamine system may affect renal function and blood pressure in the diseased animals.

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