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# Interaction of atrial natriuretic peptide, urodilatin, guanylin and uroguanylin in the isolated perfused rat kidney

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**Abstract**

*Escherichia coli* heat-stable enterotoxin (STa), guanylin and uroguanylin are novel natriuretic and kaliuretic peptides that bind to and activate membrane guanylate cyclase (GC) receptors such as GC-C and OK-GC that are expressed in the kidney and intestine. Atrial natriuretic peptide (ANP) and its renal form (urodilatin, UROD) elicit natriuretic effects by activation of a different membrane guanylate cyclase, GC-A. Experiments were done in perfused rat kidneys to search for possible synergistic interactions between ANP, UROD, guanylin and uroguanylin on renal function. Pretreatment with ANP (0.03 nM) enhanced guanylin (0.19 μM) natriuretic activity (%$ENa^+$; from 18.5±4.25 to 31.5±1.69, $P<0.05$, 120 min) and its kaliuretic activity (%$EK^+$; from 24.5±4.43 to 50.6±3.84, $P<0.05$, 120 min). Furthermore, ANP increased the natriuretic (29.05±3.00 to 37.8±2.95, $P<0.05$, 120 min) and kaliuretic (from 33.2±3.52 to 42.83±2.45, $P<0.05$, 120 min) responses of perfused kidneys treated with low-dose (0.06 μM) uroguanylin. In contrast, ANP clearly inhibited the uroguanylin-induced (0.31 μM) increase in %$ENa^+$ (from 35.9±2.37 to 14.8±1.93, $P<0.05$, 120 min), and in %$EK^+$ (from 51.0±4.43 to 38.8±3.61, $P<0.05$, 120 min). UROD (0.03 nM) also enhanced the guanylin-induced natriuresis (to %$ENa^+$=31.0±1.93, $P<0.05$, 120 min) and kaliuresis (to %$EK^+$=54.2±3.61, $P<0.05$, 120 min), and inhibited the %$ENa^+$ of uroguanylin (0.31 μM) to 17.9±1.67 as well as its %$EK^+$ to 24.3±3.13 (both at 120 min, $P<0.05$). The synergism between ANP and UROD with either guanylin or uroguanylin at sub-threshold doses and the unexpected antagonism between ANP and UROD with uroguanylin at a pharmacological dose point to possible interactions between natriuretic peptide receptor (NPR) and uroguanylin/guanylin receptor signaling pathways. The interactions herein described may play a contributory role in the regulation of kidney function in many pathophysiological states, such as in the saliuresis following ingestion of salty meals.


*Keywords:* Atrial natriuretic peptide; Urodilatin; Guanylin; Uroguanylin; Guanylate cyclase; Cyclic GMP; Natriuresis; Kidney

## 1. Introduction

The guanylin family of peptides consists of three endogenous peptides that are similar in primary structure and have biological activity comparable to *Escherichia coli* heat-stable enterotoxin (STa), a peptide that is implicated in the mechanism of traveler's diarrhea [1–3]. Guanylin was the first ST-like peptide to be isolated from the intestine [1]. Guanylin mRNAs are found in many mammalian tissues, including the kidney, uterus/oviduct, trachea, intestine, brain and adrenal medulla [4]. Guanylin and STa both activate intestinal guanylate cyclase-C (GC-C), and elicit 3′–5′ cyclic guanosine monophosphate (cGMP) accumulation in the intestinal mucosa and in the T84 human colon carcinoma cell line [5]. The increase in cGMP in target cells results in activation of the transepithelial secretion of chloride and bicarbonate anions, which serves as the driving force for fluid secretion into the intestinal lumen [2,5].

Uroguanylin was isolated initially from opossum urine and subsequently from human and rat urine [2,3]. Similar to guanylin, uroguanylin increases the levels of cGMP in T84 cells and stimulates both chloride and bicarbonate secretion in enterocytes via cGMP-mediated activation of the cystic fibrosis transmembrane conductance regulator (CFTR) protein located in apical plasma membranes [2,6]. Uroguanylin is expressed at the highest levels in the intestine, but mRNAs are also produced by the stomach, heart, kidney, and brain [3].

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Fonteles and coworkers recently reported that guanylin and uroguanylin have natriuretic and kaliuretic activities in the perfused rat kidney similar to those observed for STa [7,8]. Uroguanylin is more potent than guanylin as a natriuretic peptide and the effects of uroguanylin on urinary sodium excretion are longer lived than guanylin. Administration of uroguanylin and STa to mice elicited increases in urine volume, sodium and potassium excretion, whereas guanylin was ineffective [6]. This provides evidence that uroguanylin may play a more important role than guanylin as a regulator of renal sodium excretion in vivo [2].

Thus, uroguanylin may have a pivotal role in fluid and electrolyte homeostasis through a novel endocrine axis involving the gastrointestinal tract, heart and kidney. The stomach and/or intestine may be able to detect the amount of salt ingested resulting in an increased release of uroguanylin into the circulation to balance dietary sodium intake and urinary sodium excretion by the natriuretic action of uroguanylin in the kidney [6].

The signaling mechanisms responsible for the actions of guanylin peptides in the mammalian kidney are not clear to date. Recently, the mRNA of the receptor guanylate cyclase C was detected throughout many segments of the rat nephron [9]. Unlike the intestine, this receptor does not appear to mediate the renal effects observed for guanylin peptides, since the GC-C −/− knockout mice retain the ability to increase salt and water excretion when treated with exogenously administered guanylin and/or uroguanylin [10]. Another renal membrane guanylate cyclase activated by guanylin peptides was recently cloned from opossum kidney and from the OK cell line, and named OK-GC [11]. Since this receptor was initially identified in a marsupial animal, it is not clear whether or not a similar receptor exists in the kidney of placental mammals. Thus, the renal membrane guanylate cyclase that accounts for the biological effects observed in the perfused rat kidney remains to be identified. However, there are numerous convincing evidence that such renal responses in large part are cGMP-dependent, as reviewed recently by Forte and others [12].

Since the recognition of a natriuretic substance in the heart by De Bold in 1981, several endogenous natriuretic peptides have been identified in humans and animals [13]. Atrial natriuretic peptide (ANP) is produced by atrial myocytes and released into blood [14], brain natriuretic peptide was isolated from both brain and heart [15] and C-type natriuretic peptide was purified from porcine brain [16]. A 32-amino acid form of ANP named urodilatin is found in human urine and has its N-terminus extended by four amino acids when compared to the circulating form of human ANP [17].

Urodilatin like ANP has diuretic and natriuretic effects and acts to activate another receptor guanylate cyclase, named guanylyl cyclase A (GC-A). When exogenously administered, urodilatin is more effective as a natriuretic and diuretic agonist than ANP [18]. This observation does not correlate with the relative affinities of ANP and urodilatin binding to kidney receptors [19]. These peptides also inhibit tubular sodium reabsorption in the isolated perfused rat kidney [20]. Several studies suggest that urodilatin rather than ANP is a physiological regulator of sodium excretion by a renal paracrine mechanism [21].

Both the atriopeptin and guanylin peptides have been implicated in the complex array of endocrine mediators that regulate

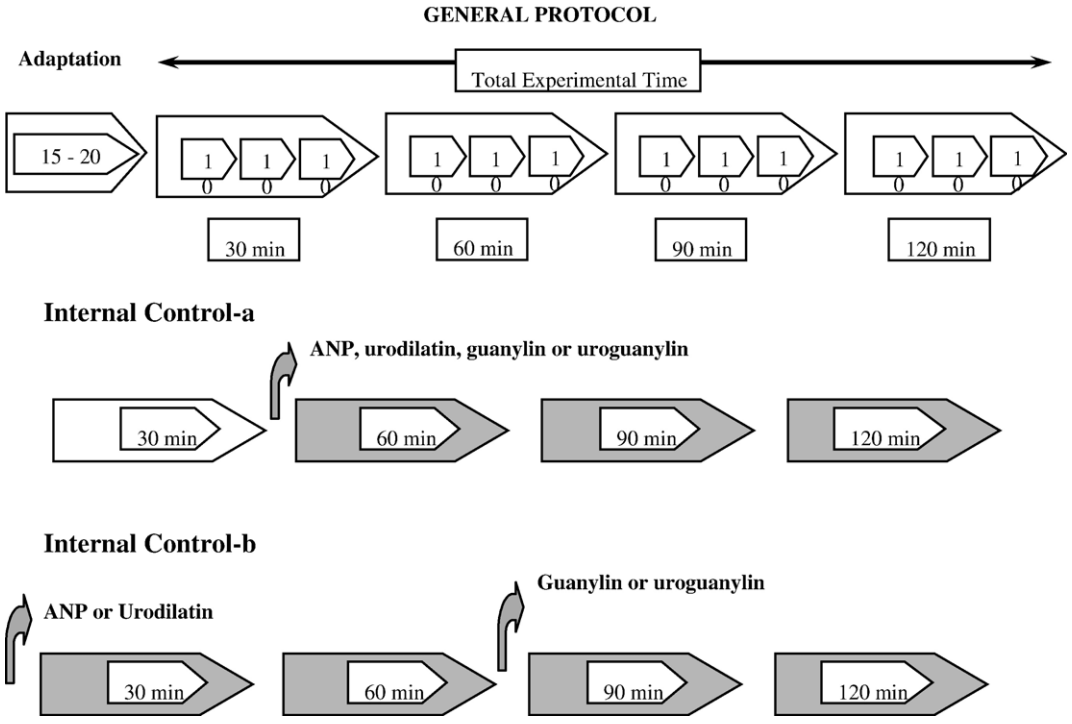


Fig. 1. The perfused rat kidney experiments followed a general protocol which consisted of a 15–20 min adaptation period before the commencement of each experiment. The total time length of each experiment was 120 min. Aliquots were collected at 10-min intervals and all data were averaged and analyzed in triplicates at 30-min intervals. In one set of experiments, a single peptide was introduced 30 min after the beginning of each experiment and functional effects were determined for 90 min. In the other group of experiments, either ANP or urodilatin was placed into the system at the beginning of each experiment, and 60 min after the introduction of ANPs, either guanylin or uroguanylin was added to the perfusion system. See Materials and methods for further details.