

Insulin-like growth factor 1 stimulates renal epithelial Na⁺ transport

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BLAZER-YOST, BONNIE L., AND MALCOLM COX. *Insulin-like growth factor 1 stimulates renal epithelial Na⁺ transport*. *Am. J. Physiol.* 255 (Cell Physiol. 24): C413–C417, 1988.—Insulin-like growth factor 1 (IGF1) stimulates vectorial Na⁺ transport in a classical model of the mammalian distal nephron, the toad urinary bladder. Net mucosal to serosal Na⁺ flux is stimulated by concentrations of IGF1 as low as 0.1 nM, and the response is maximal at 10 nM. Na⁺ transport increases within minutes of the serosal addition of IGF1, reaches a maximum in 2–3 h, and is sustained for at least 5 h. Neither the initial nor the sustained response to IGF1 is dependent on new protein synthesis. The IGF1 response is inhibited by a concentration of amiloride (10⁻⁵ M) that is known to specifically block the conductive apical Na⁺ channel but that has little effect on the Na⁺-H⁺ antiporter. Further studies will be necessary to establish a role for this growth factor in normal renal epithelial function, but it is possible that the natriferic and growth-stimulatory effects of IGF1 are intimately related.

toad urinary bladder; vectorial sodium transport

INSULIN-LIKE GROWTH FACTOR 1 (IGF1) is one of a family of endogenous growth-promoting polypeptides that appear to modulate the growth of many different tissues (2, 11, 30). A relationship between IGF1 and renal growth was evident as early as 1957 when renal hypertrophy was identified as a manifestation of acromegaly (13), a condition characterized by hypersecretion of growth hormone and a secondary increase in circulating IGF1 levels. Renal hypertrophy also occurs in compensation for the loss of renal tissue (9) and has, likewise, been related to IGF1. In unilaterally nephrectomized rats, IGF1 concentrations are increased in the remaining kidney (25). Because IGF1 mRNA levels are also increased (7), the higher tissue IGF1 levels are due, at least in part, to autocrine production of this growth factor. Interestingly, renal IGF1 synthesis is not accompanied by increased circulating IGF1 levels (25) presumably because the growth factor is utilized at or near its site of production. In this regard, specific IGF1 receptors have been demonstrated in canine proximal tubular membrane preparations (15).

A role for IGF1 in normal renal growth is more speculative. Hormone-supplemented, serum-free media have been described for the maintenance of renal epithelia in primary or continuous culture (3, 4, 17, 27, 27). These defined media are devoid of IGF1 but contain micromolar concentrations of insulin. The requirement for such high

concentrations of insulin suggests that the hormone may be acting through IGF1 receptors (9). Insulin and IGF1 receptors are structurally and functionally similar, and considerable crossover between IGF1 and insulin binding has been reported (11, 23, 30).

The cellular mechanisms by which IGF1 modulates growth are poorly defined, but changes in ion flux and/or intracellular pH are early hallmarks of the action of many growth factors (10, 14, 21, 24, 28). Growth factor activation of the Na⁺-H⁺ antiporter is a widespread phenomenon in many cells (14, 21, 24). In primary cultures of rabbit proximal tubular cells, medium insulin concentrations in the micromolar range produce cellular hypertrophy, an effect associated with increased Na⁺-H⁺ antiporter activity (10). However, in cultured monkey renal epithelial cells (BSC-1), transient alterations in intracellular Na⁺ and/or K⁺ concentrations, rather than alterations in intracellular pH, appear to be the critical determinant of cell growth (28). Although these observations are suggestive, it is clear that a definitive link has yet to be forged between growth factor-related alterations in ion flux and cell growth. Moreover, whether IGF1-related alterations in ion flux in epithelia are translated into changes in vectorial ion transport has not been directly examined.

The urinary bladder of the toad, *Bufo marinus*, is a well-characterized model renal epithelium (19, 20) that has been extremely useful in exploring the cellular mechanisms of action of a variety of natriferic hormones, most notably aldosterone (12, 20) and vasopressin (20). Interestingly, insulin is also known to enhance vectorial Na⁺ transport in this tissue (5, 29) as well as in a variety of other renal epithelia (6, 8, 16). However, for the most part, micromolar concentrations of insulin have been utilized. In the toad urinary bladder, the effective natriferic concentration of porcine insulin has been reported to be in the micromolar range (29), whereas our own studies have shown that insulin is effective at nanomolar concentrations (5, unpublished observations). The reasons for this discrepancy are not clear. However, it is unlikely that the requirement for suprapharmacological doses results from the use of mammalian (porcine) insulin in amphibian tissue. Muggeo et al. (22) have examined the specificity of a series of phylogenetically diverse insulins for specific receptors in mammals, birds, amphibia, and bony fish. The receptor has been remarkably conserved during evolution. In fact, this receptor

appears to have been better conserved than the hormone itself. For example, the binding of porcine insulin to human and amphibian receptors is virtually indistinguishable (22). Therefore, effects mediated by the binding of insulin to a specific receptor would likely be manifested at nanomolar concentrations. A requirement for micromolar doses suggests either hormone degradation or, alternatively, that the natriuretic effect may result from crossover binding to homologous receptors such as the IGF1 receptor.

In this communication, we report the novel finding that nanomolar concentrations of IGF1 stimulate Na⁺ transport in toad urinary bladders. The natriuretic effect is evident within minutes, reaches a maximum in 2–3 h, and is sustained for at least 5 h. New protein synthesis is not required for this sustained response. IGF1-stimulated Na⁺ transport is inhibited by a concentration of amiloride (10⁻⁵ M) that is known to inhibit the apical Na⁺ channel but that is unlikely to affect the Na⁺-H⁺ antiporter.

MATERIALS AND METHODS

Animals and chemicals. Female toads (*Bufo marinus*) from the Dominican Republic were obtained from National Reagents (Bridgeport, CT). IGF1 was obtained from Amgen Biological (Thousand Oaks, CA). Amiloride was a generous gift from Merck Sharp & Dohme (West Point, PA). Aldosterone, actinomycin D, and cycloheximide were obtained from Sigma Chemical (St. Louis, MO).

Electrophysiology. Toads were killed by double pithing. Urinary bladders were surgically removed and placed in aerated, Tris-buffered Ringer solution containing (in mM): 5.0 tris(hydroxymethyl)aminomethane (Tris), 0.6 KH₂PO₄, 3.5 Na₂HPO₄, 3.0 KCl, 10 dextrose, 103 NaCl, and 0.45 CaCl₂ (pH 7.95 ± 0.05, osmolality 225 ± 5 mosmol/kg H₂O). Each hemibladder was mounted as a sheet in a modified Ussing chamber to provide two quarter bladders. Thus four matched quarter bladders were obtained from each animal. Calomel reference electrodes were used for the measurement of transepithelial potential difference. Current was delivered from an automated power source. The amount of current required to clamp the potential difference at zero [the short-circuit current (SCC)] was used as a measure of net Na⁺ transport (5, 19). IGF1, actinomycin D, and cycloheximide were added to the serosal medium; amiloride was added to the mucosal medium. In each experiment, one or two quarter bladders served as controls (C), the remaining quarter bladders served as experimental tissues (E), and received IGF1 and/or other test agents as appropriate.

Data presentation. The change in SCC in experimental relative to control tissues (Δ SCC) was calculated using the following formula. Δ SCC = [SCC(*t*) - SCC(0)]_E - [SCC(*t*) - SCC(0)]_C, where SCC(*t*) is SCC at time *t* after IGF1 addition and SCC(0) is SCC at the time of IGF1 addition. Values greater than zero indicate an increase in Na⁺ transport in the experimental tissue as compared with its matched control. Comparisons were performed

using Student's *t* test for paired values; values of *P* < 0.05 were considered significant.

RESULTS

A representative experiment illustrating the Na⁺ transport response to IGF1 (10 or 30 nM) is shown in Fig. 1. Within minutes after the serosal addition of IGF1, net Na⁺ transport (monitored as SCC) increases. A new steady state is reached in 2–3 h, and the response is sustained for at least 5 h. Basal, as well as IGF1-stimulated, Na⁺ transport is completely inhibited by a low (10⁻⁵ M) concentration of the epithelial apical plasma membrane Na⁺ channel blocker, amiloride. In two additional experiments (data not shown), there was also no difference in the responses to 10 and 30 nM IGF1. Thus 10 nM appears to be the maximum effective natriuretic concentration of the growth factor. Cost considerations prevented more extensive evaluations of concentrations >10 nM.

The concentration dependence of IGF1-stimulated Na⁺ transport is illustrated in Fig. 2. At a maximal concentration (10 nM), the increase in Na⁺ transport is evident within 5 min after IGF1 addition. At submaximal concentrations (1 and 0.1 nM), the initial increase in Na⁺ transport and the new steady state achieved are proportionally lower. At all three concentrations, the natriuretic response is sustained for at least 5 h.

The role of new protein synthesis in IGF1-stimulated Na⁺ transport was examined in two separate series of experiments. Actinomycin D, an inhibitor of DNA-dependent RNA synthesis, had no effect on the natriuretic response to IGF1 (Fig. 3). The concentration of actinomycin D employed (10 μg/ml) is the highest that could be utilized without a significant inhibitory effect on basal Na⁺ transport. This concentration of actinomycin D is known to inhibit new protein synthesis in toad urinary

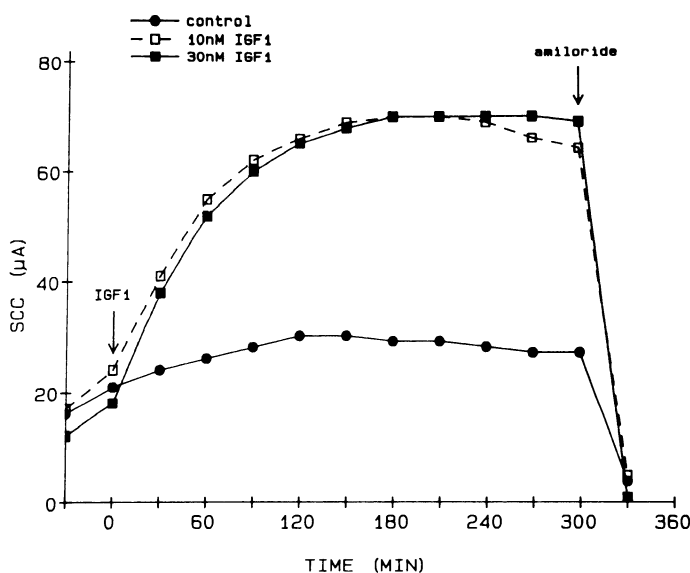


FIG. 1. Effects of insulin-like growth factor 1 (IGF1) on short-circuit current (SCC). IGF1 (10 or 30 nM) was added to experimental quarter bladders at time 0; control quarter bladder received vehicle alone. Amiloride (10⁻⁵ M) was added to all 3 quarter bladders at time = 300 min. This is a representative experiment that utilizes paired tissues from a single animal.

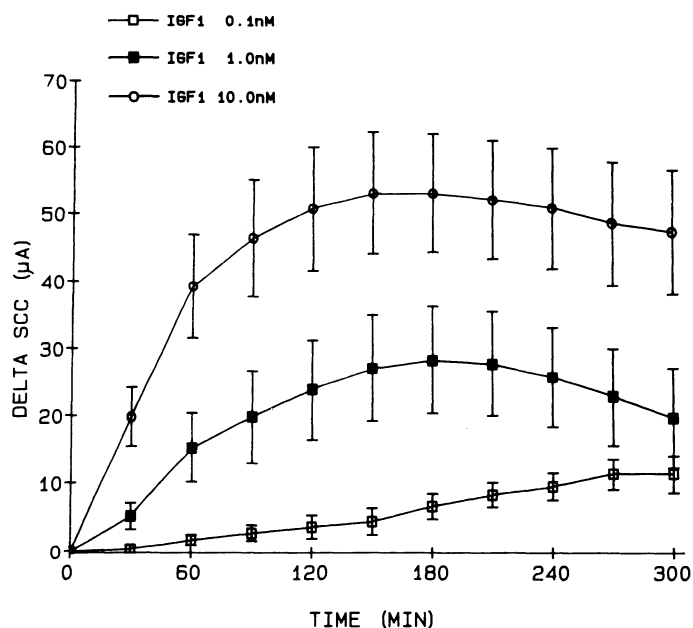


FIG. 2. Insulin-like growth factor 1 (IGF1) concentration-response relationship. IGF1 was added to experimental bladders at time 0; matched control bladders received vehicle alone. Δ SCC was calculated as described in text. Each point represents means \pm SE for 17 (10 nM), 9 (1 nM), or 9 (0.1 nM) observations. Response to 1.0 nM IGF1 was significantly less ($P < 0.05$) than that to 10 nM IGF1 at each time point and was significantly greater than that to 0.1 nM IGF1 at each time point up to and including 240 min. SCC, short-circuit current.

bladders (12) and in the present studies (data not shown) was shown to eliminate aldosterone-stimulated Na⁺ transport, a process that has been well documented to be dependent on new protein synthesis (12, 20). Cycloheximide, an inhibitor of mRNA translation, also had no effect on IGF1-stimulated Na⁺ transport (Fig. 4). The concentration of cycloheximide employed (1 μ g/ml) had no effect on basal Na⁺ transport and has previously been

shown to inhibit new protein synthesis in toad urinary bladders (5).

DISCUSSION

Studies of acromegaly (13) and of compensatory renal hypertrophy (9) have suggested a link between IGF1 and renal growth. More recently, the kidney has been shown to synthesize IGF1 (7, 25) and to have specific receptors for this growth factor in proximal tubular cells (15). Thus IGF1 is likely to have a role in normal renal growth and/or function, at least in the proximal tubule. Whether IGF1 affects other segments of the nephron is, however, unknown. Moreover, although growth factors such as IGF1 are known to modulate intracellular ion concentrations (10, 11, 14, 21, 28), their effects on vectorial ion transport in renal epithelia have not been examined. Therefore, we examined the effects of IGF1 on vectorial Na⁺ transport in a classical model of the mammalian distal nephron (19, 20), the toad urinary bladder.

IGF1 enhances net mucosal to serosal Na⁺ flux in this model renal epithelium. Vectorial Na⁺ transport is stimulated by concentrations of IGF1 as low as 0.1 nM, and the response is maximal at \sim 10 nM IGF1. This concentration-response range is consistent with the postulate that the natriuretic response is mediated through interaction with a specific receptor. For example, half-maximal specific binding of ¹²⁵I-labeled IGF1 to canine proximal tubular cell basolateral membranes occurs between 1 and 10 nM (15).

Na⁺ transport increases within minutes of the addition of a maximal concentration (10 nM) of IGF1, and the increased rate of Na⁺ flux is sustained for at least 5 h. Because of the rapidity of the response, it is unlikely that the initial increase is dependent on new protein synthesis. However, because other hormones that produce sustained increases in Na⁺ transport in renal epithelia (e.g., aldosterone) do so by way of induction of

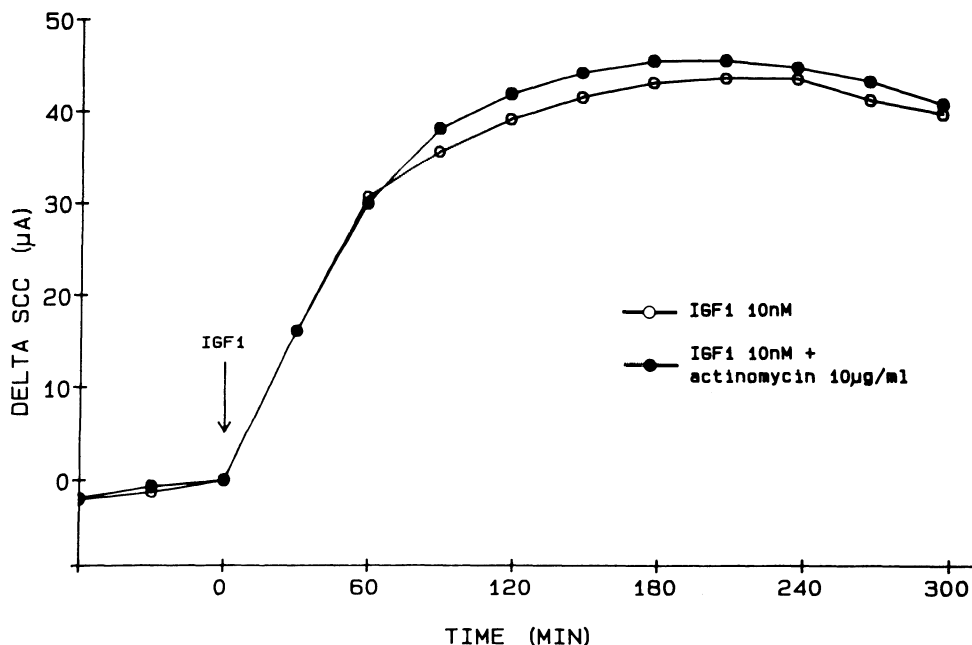


FIG. 3. Effect of actinomycin D on insulin-like growth factor 1 (IGF1)-stimulated short-circuit current (SCC). Actinomycin D (10 μ g/ml) was added to 2 of 4 matched quarter bladders 1 h before addition of IGF1; at time 0 IGF1 was added to 1 quarter bladder that had received actinomycin and to one that had not. Each point represents mean of 6 experiments. Standard errors are excluded for sake of clarity. Actinomycin D had no effect on IGF1 response.

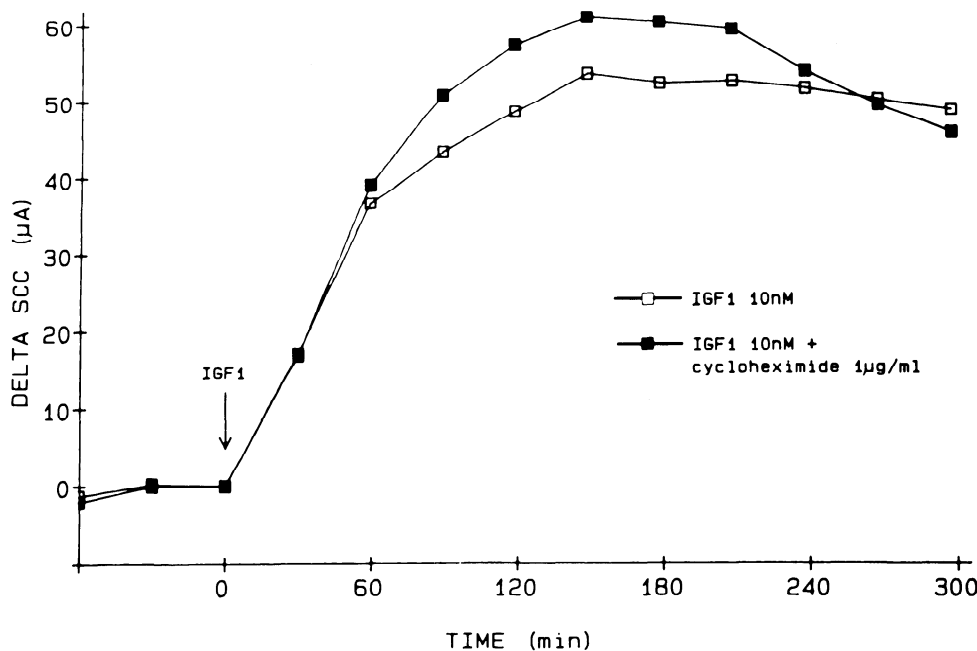


FIG. 4. Effect of cycloheximide on insulin-like growth factor 1 (IGF1)-stimulated short-circuit current (SCC). Cycloheximide (1 $\mu\text{g}/\text{ml}$) was added to 2 of 4 matched quarter bladders 1 h before addition of IGF1; at time 0, IGF1 was added to one quarter-bladder that had received cycloheximide and to one that had not. Each point represents mean of 4 experiments. Standard errors are excluded for sake of clarity. Cycloheximide had no effect on IGF1 response.

specific new "effector" proteins (12), we examined the role of new protein synthesis in the sustained response to IGF1. Although actinomycin D inhibited aldosterone-stimulated Na^+ transport, this inhibitor had no effect on IGF1-stimulated Na^+ transport. However, recent studies in BALB/c3T3 cells have shown that a specific subset of IGF1-induced proteins are insensitive to actinomycin D (31). The genes coding for these proteins are constitutively transcribed, and it appears that IGF1 regulates the stability of their mRNA transcripts. This possibility was examined using cycloheximide, which would block the translation of growth factor-stabilized mRNA. Cycloheximide had no effect on the natriuretic response to IGF1. Thus IGF1-stimulated Na^+ transport is not dependent on new protein synthesis.

The cellular site at which IGF1 exerts its natriuretic effect is unknown. In the Koefoed-Johnson and Ussing "two-barrier" model of epithelial transport (18), Na^+ is thought to passively transverse the apical plasma membrane through specific channels and then to be actively pumped across the basolateral plasma membrane by the ubiquitous $\text{Na}^+-\text{K}^+-\text{ATPase}$. Within the framework of this model, IGF1 could modulate transcellular Na^+ flux at two distinct sites: 1) the permeability of the apical membrane could be altered to allow increased Na^+ influx, or 2) the activity of the basolateral Na^+ pump could be altered either by increasing the intrinsic activity of each pump or by increasing the number of functioning pumps.

Previous studies that examined IGF1-related changes in epithelial ion transport have not examined vectorial Na^+ transport in tissues where apical membrane Na^+ flux is associated with a conductive Na^+ channel rather than a Na^+-H^+ antiporter. Proximal tubular apical membrane Na^+ flux is associated with the Na^+-H^+ antiporter, whereas Na^+ flux in the apical membrane of the distal nephron (and toad urinary bladder) is associated with a conductive Na^+ channel (1). In our studies, a concentration of amiloride (10^{-5} M) that selectively blocks the

conductive apical Na^+ channel completely abolished the natriuretic response to IGF1. Although these data do not define the precise mechanisms by which IGF1 modulates vectorial Na^+ transport, it seems clear that a functional conductive apical Na^+ channel is crucial to IGF1-stimulated Na^+ transport.

In summary, we have demonstrated that nanomolar concentrations of IGF1 stimulate Na^+ transport in a classical "high-resistance" renal epithelium, the toad urinary bladder. The physiological relevance of this observation remains to be determined and further studies will be necessary to establish a role for IGF1 in renal function. An interesting possibility is that the increase in vectorial Na^+ transport may transiently increase the intracellular Na^+ concentration, thereby providing an intracellular signal for augmented growth as has been suggested in the kidney epithelial cells of the BSC-1 cell line (28). Another possibility is that the natriuretic effect of IGF1 may simply reflect one mechanism by which the distal nephron responds to the need for increased Na^+ reabsorption that characterizes the growing kidney. Finally, it is possible that IGF1 elicits a cellular response that stimulates growth and Na^+ transport through independent, and as yet undefined, pathways.

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