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TESTOSTERONE INDUCES A RAPID STIMULATION OF ENDOCYTOSIS, AMINO ACID AND HEXOSE TRANSPORT IN MOUSE KIDNEY CORTEX

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Testosterone induced a rapid (<1 min) stimulation of endocytosis, amino acid and hexose transport, measured by the temperature-sensitive uptake of HRP, ^{14}C-AIB and ^{3}H-DG, in mouse kidney cortex slices. The hormonal increment in uptake persisted for at least 60-120 min, showed time-, energy-, and Na^{+}-dependence, and varied with substrate and testosterone concentration. Testosterone was maximally effective at 10^{-8} to 10^{-7} M. Peroxidase histochemistry indicated that the hormonal increase in HRP uptake is restricted to proximal tubules. Testosterone was more effective than DHT, whereas cyproterone acetate, androsterone and dexamethasone had little or no stimulating effect on this uptake. Kidney slices from androgen-insensitive tfm/Y mice did not respond to testosterone. The rapid increase in endocytosis, amino acid and hexose transport may represent a direct, receptor-mediated response of the surface membrane of target cells to testosterone.

Testosterone elicits a number of phenotypic effects in mouse kidney that are receptor-mediated and culminate in a substantial trophic response (1,2). We recently showed that the androgenic response in mouse kidney involves augmented activity of the lysosomal-vacuolar system in proximal tubule cells. This is manifested morphologically as enhanced autophagy, an accumulation of enlarged, membrane-packed lysosomes (myeloid bodies), and exocytosis of these lysosomes into the tubule lumen; and biochemically in elevated kidney activities of numerous lysosomal hydrolases and a striking lysosomal enzymuria and proteinuria (3,4). Testosterone also induces an early

Abbreviations: [^{3}H]DG, 2-deoxy-D-[1-^{3}H]glucose: [^{14}C]AIB, α-[1-^{14}C]-aminoisobutyric acid; HRP, horseradish peroxidase; BSS, balanced salt solution; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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(<5 min) decrease in the equilibrium density, enzyme latency and membrane stability of a population of kidney lysosomes resulting from enhanced pinosome-lysosome fusion (5-7; and unpublished data). We now report that testosterone rapidly stimulates endocytosis, hexose and amino acid transport in mouse kidney cortex.

Materials and Methods

[^{3}H]DG was obtained from Amersham, Arlington Heights, IL. [^{14}C]AIB and Aquasol were purchased from New England Nuclear, Boston, MA. HRP (Type II) and steroids came from Sigma Chemical Co., St. Louis, MO. Female A/J mice were obtained from Jackson Labs., Bar Harbor, ME. Androgen-insensitive tfm/Y mice and normal female littermates were provided by Dr. Leslie P. Bullock. Kidneys were decapsulated and placed in chilled BSS containing (in mM): NaCl, 122; KCl, 5; $CaCl_2$, 2.7; $MgSO_4$, 1.2; Na_2HPO_4, 2.6; KH_2PO_4, 0.6; Hepes buffer, 39 (pH 7.4). Surface cortex slices ~300 μ thick were placed in 25 ml-beakers (4 slices ~30 mg wet weight) in 2.5 ml of BSS containing 6 mM glucose. Steroids were added in 25 μl of 5% ethanol; controls received solvent. HRP (1 mg/ml) was added to the incubation medium to quantify endocytosis, [^{14}C]AIB (0.1 mM, 4 μCi/ml) to measure amino acid transport, and [^{3}H]DG (0.1 mM, 1 μCi/ml) to measure hexose transport. Incubations were carried out at 30 or 37° C under 95% O_2-5% CO_2 in a shaking incubator. After incubation the tissue samples were placed on ice, rinsed 3 times in BSS, lysed in water and assayed for protein (8), HRP (9) and radioactivity (by scintillation spectrometry). Corrections for nonspecific uptake were made by subtracting the amount of tracers taken up by slices at 0° C. For cytochemical observation of HRP uptake, tissue specimens were fixed in cold 1.5% glutaraldehyde with 0.1 M sod. cacodylate, pH 7.4, incubated for peroxidase activity (10) and processed for light and electron microscopy.

Results and Discussion

Endocytosis. The temperature-sensitive uptake of HRP into kidney slices was linear over a 1-2 h period (Fig. 1). This uptake varied linearly with solute concentration (0.5 - 5 mg/ml) consistent with bulk volume endocytosis. In histochemical preparations proximal tubules occupied approximately 60-70% of the total area of cortex slices. During incubation of slices with HRP (5 mg/ml) at 0° C, peroxidase reaction product was not detectable intracellularly (except in sparse peroxisomes) although some product was associated with peritubular membranes. Incubation of slices at 30° or 37° C for up to 60 min resulted in a time-dependent increase in intracellular enzyme product largely restricted to endosomes and lysosomes in proximal tubules.

Testosterone increased the endocytosis rate, i.e., temperature-sensitive uptake, of HRP by 45-100% (Fig. 1). This increase appeared within one

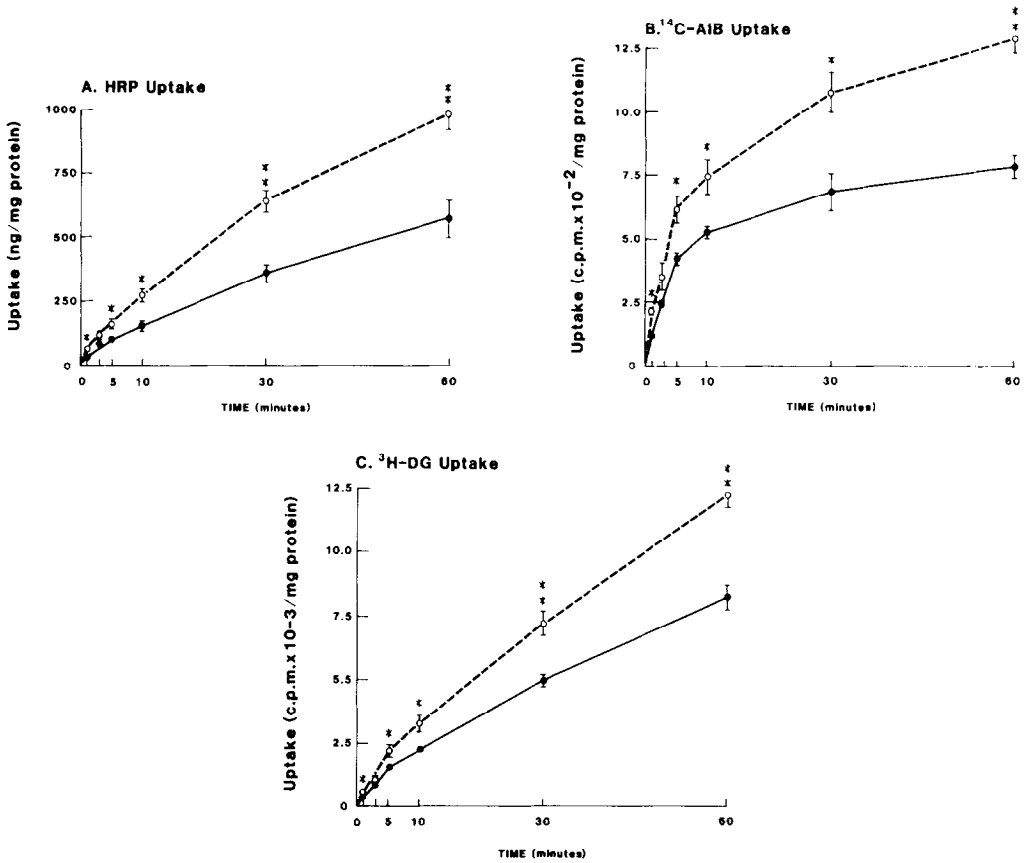

Fig. 1. Effect of testosterone on the time-course of HRP, AIB and DG uptake by mouse kidney cortex in vitro. Cortex slices were preincubated in BSS with 6 mM glucose for 5 min at 37° C. At 0 time slices were transferred to fresh medium containing HRP (5 mg/ml), [^{14}C]AIB (.1 mM, 4 μCi/ml), [^{3}H]DG (.1 mM, 1 μCi/ml) and 10^{-8} M testosterone (open circle) or no hormone (closed circle). Tissue slices were removed for study at each time point of incubation starting at 1 min. Data were corrected for nonspecific uptake as described in the text. Results are means ± SEM (n=3). *,**,***: p<.05, .01, .001 (Student's t test).

min and persisted for at least 2 h. The increase in HRP uptake varied with testosterone concentration and was maximal at 10^{-8} to 10^{-7} M (Fig. 2). In peroxidase-stained preparations testosterone-induced endocytosis was confined to the proximal tubules (detailed observations will be published separately). Sodium azide (0.5, 2.5 mM) and dinitrophenol (0.3, 1.5 mM), which inhibit ATP production by the respiratory pathway, inhibited HRP uptake in testosterone-stimulated slices. Omission of added glucose or equimolar replacement of NaCl with choline chloride abolished the hormonal increment in HRP uptake without affecting basal uptake (data not shown). These findings indicate that the hormonal response is energy- and sodium-dependent.

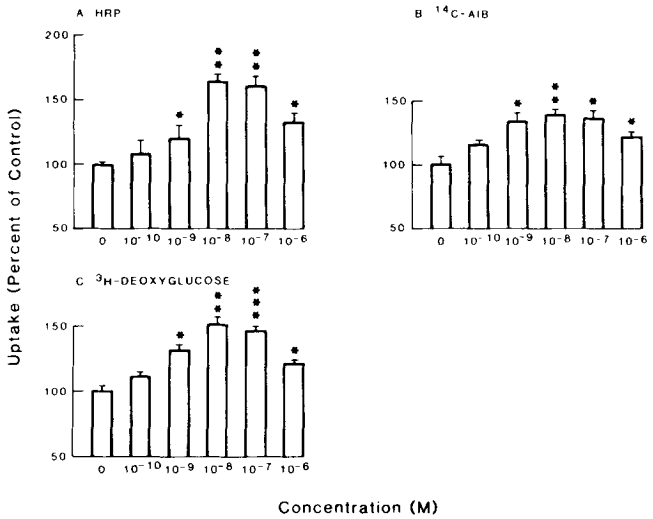

Fig. 2. Effect of testosterone concentration on HRP, AIB and DG uptake by mouse kidney cortex. After a 5 min preincubation in BSS with 6 mM glucose, cortex slices were placed in fresh medium containing [^{14}C]AIB, [^{3}H]DG, HRP (1 mg/ml) and various concentrations of testosterone. AIB and DG uptake was measured after a 5 min incubation, and HRP uptake after a 60 min incubation at 37° C. Hormone-free control uptake values were: HRP, 44 ± 4 ng/mg/h; [^{14}C]AIB, 760 ± 46 c.p.m./mg/5 min; [^{3}H]DG, 4505 ± 200 c.p.m./mg/5 min. Results are means ± SEM (n=3). Additional details are given in the legend to Fig. 1 and in the text.

Steroid specificity was assessed by comparing the effects of several steroids differing in androgenic potency on the rate of endocytosis (Fig. 3). DHT was less effective than testosterone; cyproterone acetate (10^{-7} M) induced only a modest stimulation, while c-androsterone and dexamethasone (10^{-6} M) (not shown) had no effect on HRP uptake. The role of specific androgen receptors was investigated in tfm/Y mice, which are deficient in functional androgen receptors (2). Testosterone had no effect on HRP uptake in cortical slices from tfm/Y mice, but augmented HRP uptake in slices from unaffected littermates (Fig. 4).

AIB and DG Uptake. The temperature-sensitive uptake of AIB and DG by kidney slices was linear for about 5 min and then gradually decreased (Fig. 1). AIB and DG uptake increased linearly with substrate concentration between 0.1 and 0.5 mM but deviated increasingly in the range of 1-100 mM. Basal AIB uptake was energy- and partially (∿50%) sodium-dependent, and was inhibited by 2 mM L-alanine; basal DG uptake was sodium-independent and inhibited by 6 mM D-glucose, but not by 6 mM L-glucose (data not shown). These

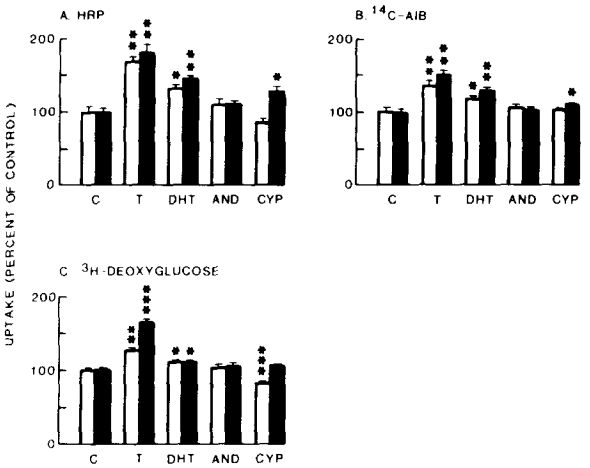

Fig. 3. Steroid specificity of the testosterone-induced uptake of HRP, AIB and DG by mouse kidney cortex. After a 5 min preincubation cortex slices were incubated in BSS with 6 mM glucose, HRP, [^{14}C]AIB, [^{3}H]DG and testosterone, DHT, c-androsterone (AND) or cyproterone acetate (CYP) at 10^{-8} M (open bars) or 10^{-7} M (closed bars). Steroid free control (C) uptake values for the 10^{-8} M and 10^{-7} M series were: HRP, 109 ± 10 and 87 ± 4.1 ng/mg/h; [^{14}C]AIB, 736 ± 38 and 927 ± 29 c.p.m./mg/5 min; [^{3}H]DG, 5440 ± 92 and 1956 ± 49 c.p.m./mg/5 min. Results are means ± SEM (n=3).

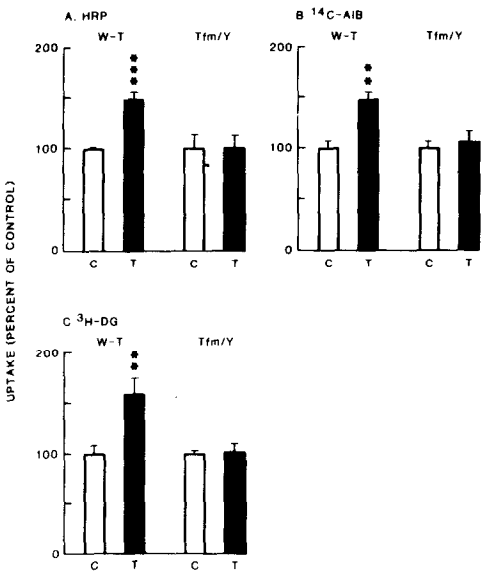

Fig. 4. Effect of testosterone on HRP, AIB and DG uptake by kidney cortex from tfm/Y mice. Cortex slices from tfm/Y mice and normal female (wild-type) (W-T) littermates were preincubated in BSS with 6 mM glucose and incubated in the same medium with HRP, [^{14}C]AIB and [^{3}H]DG without (C) or with 10^{-8} M testosterone (T). Control W-T and tfm/Y uptake values were: HRP, 141 ± 2 and 128 ± 16 ng/mg/h; [^{14}C]AIB, 456 ± 33 and 429 ± 28 c.p.m./mg/5 min; [^{3}H]DG, 2045 ± 143 and 2317 ± 62 c.p.m./mg/5 min. Results are means ± SEM (n=3).

results support the inference that sodium-dependent AIB uptake reflects the initial rate of amino acid transport via the A (alanine-preferring) system (11); and DG uptake occurs through a stereospecific, saturable transport system that mediates D-glucose transport.

Testosterone increased AIB and DG transport in cortical slices by 30-65% (Fig. 1). This increase was detectable within one min after addition of hormone and was still evident after 2 h of incubation. The hormonal increment in uptake was observed over a broad range of AIB and DG concentrations (0.1 - 100 mM) (not shown). Hormonal stimulation of AIB and DG transport varied with testosterone concentration and peaked at 10^{-8} to 10^{-7} M (Fig. 2). The hormonal increment in AIB and DG uptake was decreased or blocked when either glucose was omitted or NaCl replaced by choline chloride (data not shown). Testosterone was more effective than DHT in stimulating AIB and DG transport, while c-androsterone, cyproterone acetate (Fig. 3) and dexamethasone (10^{-6} M) (not shown) had little or no stimulating effect on this transport. Testosterone did not affect AIB or DG uptake in cortical slices from tfm/Y mice, but stimulated such uptake in slices from unaffected littermates (Fig. 4).

Our experiments have shown that physiological concentrations of testosterone (1-10 nM) (12) induce a rapid (<1 min) increase in endocytosis, hexose and amino acid transport in mouse kidney cortex. It is clear from the histochemical observations of HRP uptake that proximal tubules are the major target for the early effects of testosterone, as they are for the long-term androgenic response, e.g., lysosomal and mitochondrial changes, increased β-glucuronidase activity (3,4).

The early androgenic response exhibits steroid specificity with testosterone being more potent than DHT. This finding is consistent with evidence indicating that testosterone has a greater binding affinity for the kidney androgen receptor than DHT, and that testosterone rather than one of its metabolites is the major androgenic effector in mouse kidney (2). Additional support for the involvement of androgen receptors comes from the

failure of tfm/Y mouse kidney cortex to display the rapid testosterone response.

It is generally believed that steroid hormones regulate target cell function by influencing RNA synthesis through the formation of steroid-receptor complexes in the cell interior (13). However, the testosterone-induced stimulation of pinocytosis, hexose and amino acid transport appears earlier (<1 min) than the increase in RNA polymerase activity and chromatin template capacity (∿15 min) in mouse kidney (2,14). Furthermore, testosterone-induced endocytosis is not initially dependent on RNA or protein synthesis (7). The present findings are more compatible with the view that the rapid testosterone response represents a direct, receptor-mediated action of the steroid on the surface membrane. Additional evidence for this view comes from subcellular fractionation studies indicating that testosterone (and its glucuronide) is transported into kidney target cells by endocytosis and compartmented within lysosomes (15, and in preparation); and that specific, saturable androgen receptors present in a plasma membrane-containing fraction may be implicated in this process (C. Barsano, A. Goldstone & H. Koenig, unpublished). It will be important in future work to correlate the early plasma membrane response with androgen binding to putative surface membrane receptors; and to define the relation of the surface membrane response to the later events in the cell interior.

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