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β-Adrenergic stimulation of Ca^{2+} fluxes, endocytosis, hexose transport, and amino acid transport in mouse kidney cortex is mediated by polyamine synthesis

(signal transduction and transmission/stimulus–response coupling/ornithine decarboxylase activation/isoproterenol)

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ABSTRACT **We recently found that the β-adrenergic agonist 1-isoproterenol evokes a rapid (<5 min) Ca^{2+}- and receptor-dependent stimulation of endocytosis, hexose transport, and amino acid transport in mouse renal cortex involving proximal tubule cells. This response is associated with increased Ca^{2+} fluxes and a mobilization of mitochondrial calcium, suggesting that stimulus–response (stimulus-"transport") coupling is mediated by cytosolic Ca^{2+}. We show here that 1 μM isoproterenol evokes a rapid (<60 sec) transient increase in the activity of ornithine decarboxylase followed by an early (<2 min) sustained increase in putrescine, spermidine, and spermine concentrations in mouse kidney cortex slices *in vitro*. Small doses of isoproterenol (down to 24 nmol/kg) elicited a rapid (<2 min) increase in polyamines *in vivo*. The ornithine decarboxylase inhibitor α-difluoromethylornithine (5 mM) suppressed the testosterone-induced increase in polyamine levels and rates of endocytosis, hexose transport, and amino acid transport, measured by horseradish peroxidase, [^{14}C]aminoisobutyric acid, and deoxy[^{3}H]glucose uptake. α-Difluoromethylornithine also blocked the isoproterenol-induced increase in ^{45}Ca influx and efflux and ^{45}Ca redistribution; 0.5 mM putrescine nullified α-difluoromethylornithine inhibition and restored the increment in polyamines, ^{45}Ca fluxes, endocytosis, hexose transport, and amino acid transport. These data implicate polyamine synthesis in isoproterenol stimulation of Ca^{2+} fluxes and membrane transport processes and support a model for signal transduction and stimulus–response coupling in which ornithine decarboxylase activation and polyamine synthesis play a pivotal role in regulating Ca^{2+} fluxes. In this model the polyamines generate local Ca^{2+} signals by stimulating Ca^{2+} influx or mobilizing intracellular calcium (or both) through a cation exchange reaction.**

In many tissues activation of cell surface receptors by hormones, neurotransmitters, and other external stimuli evokes changes in Ca^{2+} fluxes, which apparently lead to an increase in cytosolic Ca^{2+}, an acknowledged mediator of numerous cell responses (1, 2). The changes in Ca^{2+} fluxes involve an inflow of extracellular Ca^{2+} through Ca^{2+} channels in the plasma membrane or an outflow of Ca^{2+} from cell organelles, or both. The molecular mechanisms by which surface agonists increase Ca^{2+} influx across the plasma membrane or regulate the outflow of Ca^{2+} from mitochondria and other cell organelles are unknown but probably involve the generation of one or more intracellular signals at the plasma membrane. However, the identity of these signals has remained elusive. Enhanced synthesis of the aliphatic polyamines putrescine, spermidine, and spermine is one of the earliest events that occur during cell growth, replication, differentiation, transformation, and virus infection (3, 4). Ornithine decarboxylase (OrnDCase), the first and rate-limiting enzyme in the polyamine synthetic pathway, is notable for its striking inducibility in response to hormones, growth stimuli, drugs, and toxins and its exceptionally short half-life (10–20 min) (3, 4). The polyamines have been implicated in the modulation of RNA, DNA, and protein synthesis (3, 4), cytomembrane (3–5) and cytoskeletal function (4, 6), and metabolic reactions as diverse as activation of phosphorylase b (7) and choline kinase (8), glucose oxidation (9), and lipolysis (9). However, the precise physiological roles and mechanism of action of the polyamines are as yet undefined.

Recently we found that the β-adrenergic agonist 1-isoproterenol evokes an early (<5 min) Ca^{2+}- and receptor-dependent stimulation of endocytosis, hexose transport, and amino acid transport in mouse kidney cortex involving the proximal tubules (10, 11). This early response is associated with increased Ca^{2+} fluxes and a mobilization of intracellular calcium and is thought to represent a direct β-adrenoreceptor-mediated action of isoproterenol on the surface membrane of target cells (10, 11). Isoproterenol stimulation of membrane transport functions exhibits an absolute requirement for a verapamil-sensitive influx of extracellular Ca^{2+} and is mimicked by the Ca^{2+} ionophore A23187, suggesting the involvement of cytosolic Ca^{2+} in stimulus–response (stimulus-"transport") coupling (11, 12). We report here that isoproterenol evokes a rapid transient increase in OrnDCase activity and a sustained increase in polyamine concentrations in mouse kidney cortex. This enhanced polyamine synthesis appears to be indispensible for the isoproterenol-mediated changes in Ca^{2+} fluxes and the attendant stimulation of endocytosis, hexose transport, and amino acid transport. An early stimulation of polyamine synthesis has been implicated in the mediation of a rapid membrane transport response to a steroid hormone testosterone (13, 14). These findings are consistent with a theory of information flow in stimulus–response coupling in which the polyamines serve as intracellular signals or messengers to increase free cytosolic Ca^{2+} by enhancing Ca^{2+} influx and mobilizing Ca^{2+} from intracellular stores via a cation exchange reaction (13, 14).

METHODS

Materials. α-[1-^{14}C]Aminoisobutyric acid ([^{14}C]AIB), ^{45}Ca, L-[3-^{3}H(n)]-ornithine (20 Ci/mmol; 1 Ci = 3.7×10^{10} Bq), and Aquasol were purchased from New England Nuclear. 2-Deoxy-D-[1-^{3}H]glucose ([^{3}H]dGlc) was from Amersham. Horseradish peroxidase (HRP, type II) and l-isoproterenol came from Sigma. Propranolol was from Ayerst Laboratories, (New York). α-Difluoromethylornithine (CHF_2-Orn) was supplied by Merrell-

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Abbreviations: OrnDCase, ornithine decarboxylase; CHF_2-Orn, α-difluoromethylornithine; AIB, α-aminoisobutyric acid; dGlc, 2-deoxy-D-glucose; HRP, horseradish peroxidase.

National Laboratories (Cincinnati, OH). Female A/J mice were purchased from The Jackson Laboratory and female C57-BL6-COX mice were from Laboratory Supply (Indianapolis, IN).

Incubations. Kidneys were rapidly excised, decapsulated, and placed in incubation medium 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 (pH 7.4), 39; glucose, 6, at 0°C. Surface cortical slices 0.3 mm thick were prepared from decapsulated kidney. Incubations were carried out in 25-ml beakers in incubation medium with appropriate additions at 37°C under 95% O_2/5% CO_2 in a shaking incubator.

Analytical Methods. Polyamines were measured by spectrophotometry of the dansylamide derivatives after separation by thin-layer chromatography (15). Protein was measured according to Lowry *et al.* (16), and OrnDCase activity was measured according to Dhurhuus (17) as described elsewhere (14).

Rates of Endocytosis, Hexose Transport, and Amino Acid Transport. Endocytosis, amino acid transport, and hexose transport were measured by monitoring HRP, [^{14}C]AIB, and [^{3}H]dGlc uptake in cortex slices as described (18).

^{45}Ca Influx, Efflux, and Distribution. Rates of ^{45}Ca influx and efflux were measured in cortex slices with selected additions as given in *Results* (12, 14). For the subcellular distribution of ^{45}Ca, cortex slices were preincubated for 30 min with ^{45}Ca (3–4 μCi/ml), rinsed, and incubated for 10 min at 37°C in fresh medium with appropriate additives as described in *Results*. Slices were homogenized in 2 ml of ice-cold 0.25 M sucrose/2 mM Hepes, pH 7.4, containing 10 μM ruthenium red, 0.1 mM La_2O_3, and 1 mM EGTA. The homogenates were fractionated by differential centrifugation into a nuclear, mitochondrial, microsomal, and soluble fraction (12, 14).

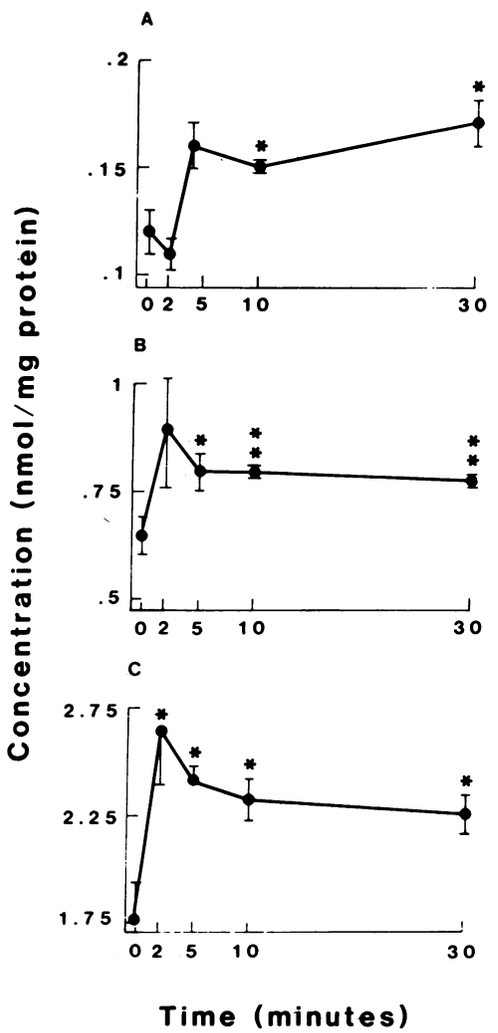

FIG. 1. Isoproterenol induces an acute increase in mouse kidney polyamines *in vivo*. (*A*) Putrescine; (*B*) spermidine; (*C*) spermine. Mice received isoproterenol (5 mg/kg intraperitoneally) under pentobarbital anesthesia. Kidneys were quickly removed at 0, 2, 5, 10, and 30 min and analyzed for polyamines. Data are means ± SEM (n = 3). *, **: $P < 0.05, 0.01$ (Student's t test).

RESULTS

OrnDCase Activity and Polyamine Concentrations. Isoproterenol, 5 μg to 5 mg/kg intraperitoneally, induced a rapid ac-

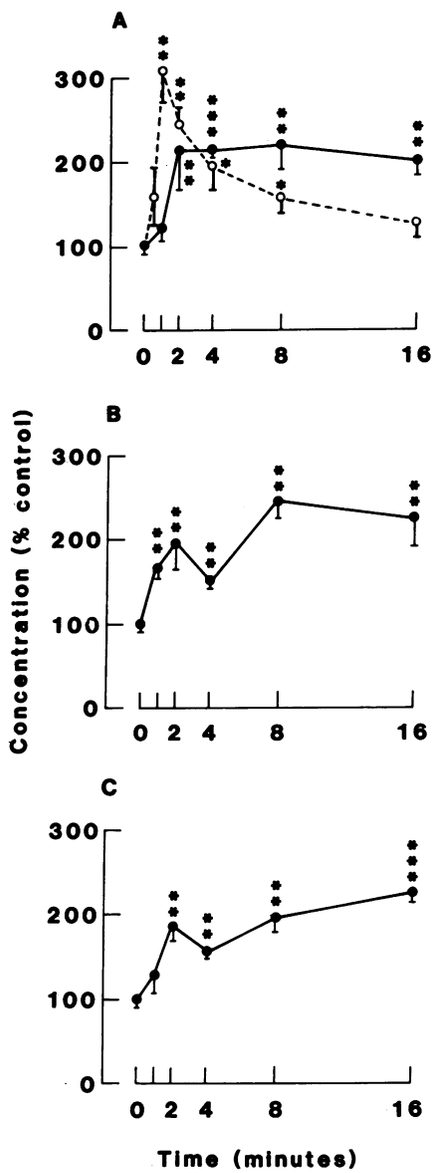

FIG. 2. Isoproterenol induces an early increase in OrnDCase activity and polyamine concentrations in mouse kidney cortex *in vitro*. (*A*) OrnDCase and putrescine; (*B*) spermidine; (*C*) spermine. Cortex slices were incubated with 1 μM isoproterenol at 37°C for various times and analyzed for OrnDCase and polyamines. Zero-time control values (mean ± SEM) were: OrnDCase, 880 ± 170 pmol/mg of protein per hr; putrescine, 0.14 ± 0.012 nmol/mg of protein per hr; spermidine, 0.44 ± 0.03 nmol/mg of protein per hr; spermine, 1.67 ± 0.13 nmol/mg of protein per hr. Data are means ± SEM. *, **, ***: $P < 0.05, 0.01, 0.001$ (vs. zero-time control).

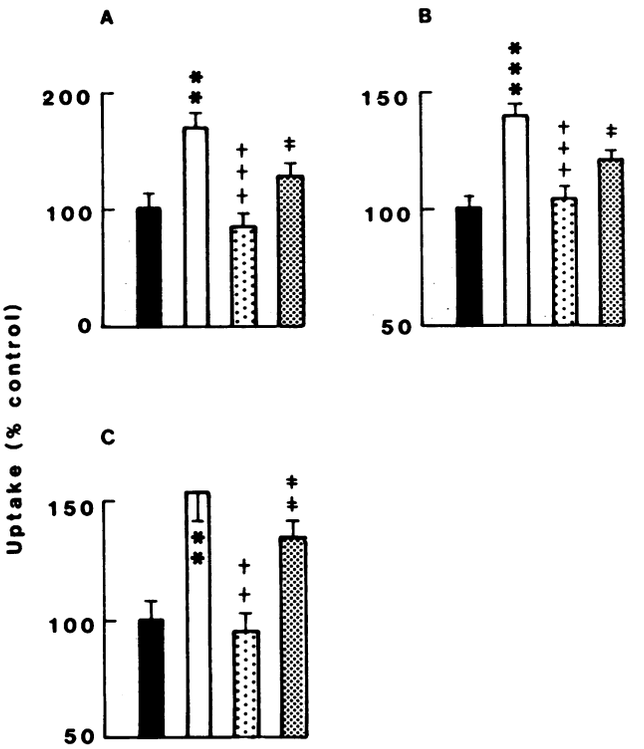

FIG. 3. CHF_2-Orn abolishes isoproterenol stimulation of endocytosis, hexose transport, and amino acid transport in kidney cortex, and putrescine reverses the CHF_2-Orn effect. Cortex slices were preincubated for 10 min at 37°C with 5 mM CHF_2-Orn, CHF_2-Orn and 0.5 mM putrescine, or no additions. Slices were transferred to fresh medium of the same composition containing HRP (*A*), [^{14}C]AIB (*B*), [^{3}H]dGlc (*C*), and 1 μM isoproterenol (□) or no hormone (■, control). ⊡, Isoproterenol and CHF_2-Orn; ⊠, isoproterenol, CHF_2-Orn, and putrescine. Control uptake values (mean ± SEM) were: HRP, 190 ± 25.5 ng/mg per hr; [^{14}C]AIB, 749 ± 40 cpm/mg per 5 min; [^{3}H]dGlc, 3,207 ± 235 cpm/mg per 5 min. Data are means ± SEM (n = 6). *, **, ***: $P < 0.05$, 0.01, 0.001 (vs. control). +, ++, +++: $P < 0.05$, 0.01, 0.001 (vs. isoproterenol). ‡, ‡‡, ‡‡‡: $P < 0.05$, 0.01, 0.001 (vs. isoproterenol and CHF_2-Orn) (key for Figs. 3–6).

cumulation of kidney polyamines *in vivo* (Fig. 1). Isoproterenol (1 μM) also elicited an accumulation of polyamines in kidney cortex slices during short incubations (Fig. 2). Significant increases in concentration of all three polyamines appeared by 2 min and persisted for at least 30 min of incubation. An abrupt rise in OrnDCase activity preceded the increase in polyamines (Fig. 2). OrnDCase activity was consistently elevated by 60–90 sec of incubation with isoproterenol and usually returned to control levels by 8–15 min. These experiments establish that an increase in OrnDCase activity and enhanced polyamine synthesis are very early events in isoproterenol-stimulated mouse kidney cortex.

Relation of Polyamine Synthesis to Membrane Transport Response. Because OrnDCase activation and polyamine accumulation develop in isoproterenol-stimulated kidney cortex before or coincident with the increase in endocytosis, hexose transport, and amino acid transport (10, 11), we explored the role of polyamines in this early plasma membrane response with the aid of CHF_2-Orn, a specific, irreversible, enzyme-activated inhibitor of OrnDCase (19). In confirmation of earlier results (10, 11), 1 μM isoproterenol induced a rapid (<5 min) stimulation of endocytosis, amino acid transport, and hexose transport (Fig. 3). CHF_2-Orn (5 mM) abolished the isoproterenol-induced increase in endocytosis, amino acid transport, and hexose transport. CHF_2-Orn also decreased basal endocytosis, amino acid transport, and hexose transport in unstimulated kidney cortex slices by about 20% (not shown). Putrescine (0.5 mM) nullified the effect of CHF_2-Orn and restored the increment in endocytosis, hexose transport, and amino acid transport. As shown earlier (14), putrescine alone evoked a similar increase in these transport processes (not shown). CHF_2-Orn inhibited the isoproterenol-stimulated increase in OrnDCase by about 70–80% and suppressed the increment in polyamines evoked by isoproterenol (Table 1). Putrescine, in the presence of isoproterenol and CHF_2-Orn, increased the tissue concentrations of all three polyamines. These data provide strong evidence for the view that polyamine synthesis is involved in the mediation of the early membrane effect of isoproterenol.

Relation of Polyamine Synthesis to Ca^{2+} Fluxes. Isoproterenol (1 μM) rapidly (<30 sec) stimulated ^{45}Ca influx (Fig. 4) and efflux (Fig. 5), decreased mitochondrial ^{45}Ca, and increased soluble ^{45}Ca (Fig. 6) in kidney cortex, as previously reported (11); 5 mM CHF_2-Orn suppressed the isoproterenol-mediated stimulation of ^{45}Ca influx during incubations of 0.5–4 min (Fig. 4). Putrescine nullified CHF_2-Orn inhibition and restored ^{45}Ca influx. CHF_2-Orn also abolished the increase in ^{45}Ca efflux elicited by isoproterenol in incubations of 0.5–20 min, whereas putrescine nullified CHF_2-Orn inhibition and restored the increment in ^{45}Ca efflux (Fig. 5). Finally, CHF_2-Orn prevented the isoproterenol-mediated decrease in mitochondrial ^{45}Ca and the increase in cytosolic ^{45}Ca, and putrescine blocked the CHF_2-Orn effect (Fig. 6). These results support the inference that polyamine synthesis is involved in the stimulation of Ca^{2+} fluxes evoked in kidney cortex by isoproterenol.

DISCUSSION

The early increase in OrnDCase and polyamine levels induced in mouse kidney cortex by isoproterenol closely resembles that

Table 1. Effect of CHF_2-Orn and putrescine on the rapid increase in kidney cortex polyamines evoked by isoproterenol

Treatment	Putrescine, nmol/mg of protein	Spermidine, nmol/mg of protein	Spermine, nmol/mg of protein
No addition	0.22 ± 0.04	0.47 ± 0.04	1.57 ± 0.14
1 μM Isoproterenol	0.31 ± 0.07	0.85 ± 0.13*	2.43 ± 0.51*
+ 5 mM CHF_2-Orn	0.22 ± 0.02	0.52 ± 0.03†	1.20 ± 0.17†
+ 5 mM CHF_2-Orn + 0.5 mM putrescine	1.32 ± 0.09‡	0.73 ± 0.03§	2.00 ± 0.02¶

Cortex slices were preincubated with 5 mM CHF_2-Orn, CHF_2-Orn and 0.5 mM putrescine, or no addition for 10 min at 37°C and then were incubated for 5 min at 37°C with or without 1 μM isoproterenol. Corrections for nonspecific uptake of putrescine were made by subtracting the 0°C uptakes. Results are means ± SEM (n = 3).

*$P < 0.05$ (vs. no addition).

†$P < 0.05$ (vs. isoproterenol).

‡$P < 0.001$ (vs. isoproterenol and CHF_2-Orn).

§$P < 0.05$ (vs. isoproterenol and CHF_2-Orn).

¶$P < 0.01$ (vs. isoproterenol and CHF_2-Orn).

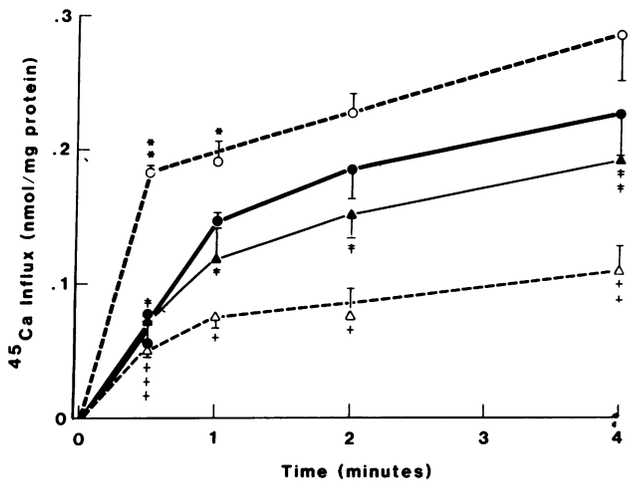

FIG. 4. CHF_2-Orn blocks the isoproterenol-induced increase in ^{45}Ca influx in kidney cortex, and putrescine reverses CHF_2-Orn inhibition. Cortex slices were preincubated with 5 mM CHF_2-Orn, CHF_2-Orn and 0.5 mM putrescine, or no agent for 10 min at 37°C and transferred to fresh medium containing ^{45}Ca (1–2 μCi/ml) with 1 μM isoproterenol (○----○), isoproterenol and 5 mM CHF_2-Orn (△----△), isoproterenol, CHF_2-Orn and 0.5 mM putrescine (▲——▲), or no agents (●——●). Data are means ± SEM (n = 3).

recently found in testosterone-stimulated kidney cortex (13, 14) but differs radically from the many examples of OrnDCase induction described in the literature in its temporal pattern. Classically the increase in OrnDCase activity virtually always occurs about 2–4 hr after the administration of a hormone or other growth stimulus (3, 4). In contrast, the early transient increase in OrnDCase activity in the isoproterenol-stimulated kidney cortex occurs within 1–2 min and returns to basal values by 8–15 min, with an apparent half-life ≈3 min. The rapidity of these changes in OrnDCase activity precludes a transcriptional or translational process in its mediation. In addition to β-adrenoreceptors and Ca^{2+}, the synthesis of prostaglandins and cAMP appears to be essential for isoproterenol stimulation of OrnDCase activity and membrane transport functions (unpublished data). Furthermore, the prostaglandins PGA_2 and PGE_2 and dibutyryl-cAMP have been shown to rapidly stimulate OrnDCase activity (20) and membrane transport functions in kidney cortex (10). Therefore, this early transient increase in OrnDCase activity likely represents a receptor-mediated, Ca^{2+}-, prostaglandin-, and cAMP-dependent activation of a latent OrnDCase via a posttranslational mechanism, possibly a phosphorylation–dephosphorylation sequence involving OrnDCase or an OrnDCase-regulating protein.

Our experiments have provided persuasive evidence that the early stimulation of OrnDCase activity and polyamine accumulation are involved in the isoproterenol-induced stimulation of Ca^{2+} fluxes, endocytosis, hexose transport, and amino acid transport in kidney cortex. These findings are consistent with the hypothesis that the polyamines serve as intracellular signals or messengers to increase the influx of extracellular Ca^{2+} across the plasma membrane and the efflux of Ca^{2+} from the mitochondria and other organelles. The resultant rise in free cytosolic Ca^{2+} triggers the coordinate increment in endocytosis, hexose transport, and amino acid transport and stimulates other Ca^{2+}- (or Ca^{2+}-calmodulin) mediated reactions at the plasma membrane and in the cell interior.

The data reported in this communication support the following cellular control system for the mediation of the early membrane response. Transduction of the β-adrenergic signal into an

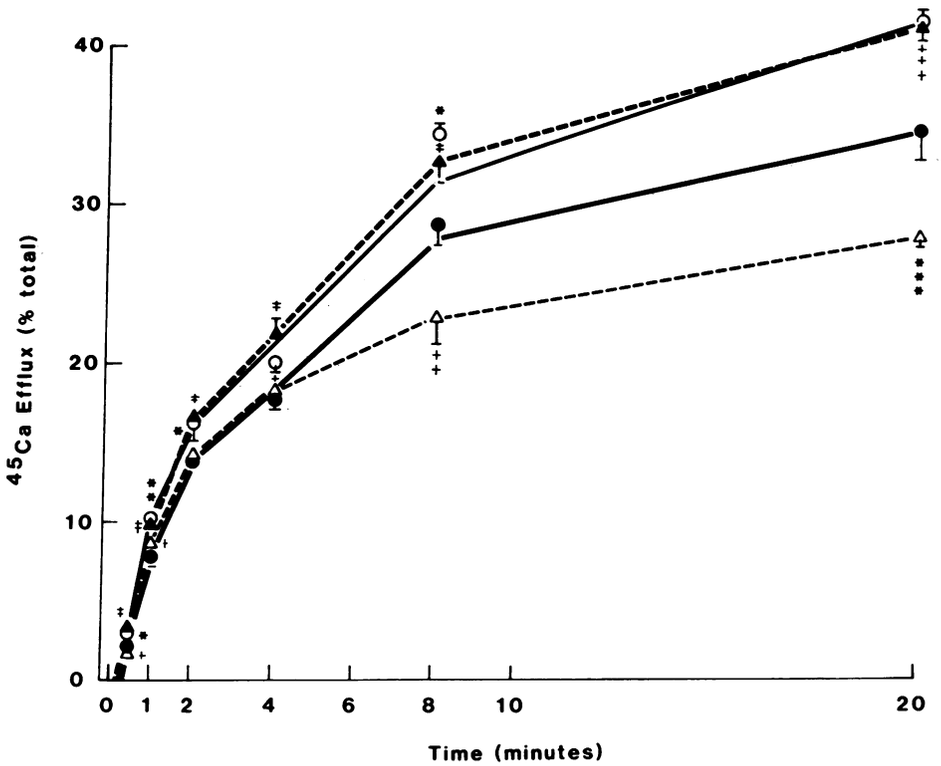

FIG. 5. CHF_2-Orn suppresses the isoproterenol-induced increase in ^{45}Ca efflux in kidney cortex, and putrescine reverses CHF_2-Orn suppression. Cortex slices were preincubated for 20 min at 37°C in incubation medium containing ^{45}Ca (≈5 μCi/mol); 5 mM CHF_2-Orn, CHF_2-Orn and 0.5 mM putrescine, or no agent was then added and the preincubation was ended 10 min later. Slices were washed in cold nonradioactive medium and incubated in fresh medium with 1 μM isoproterenol (○---○), isoproterenol and CHF_2-Orn (△---△), isoproterenol, CHF_2-Orn, and putrescine (▲——▲), or no additions (●——●, control) for 10 min. ^{45}Ca effluxes are expressed as % of total ^{45}Ca (medium and tissue). Data are means ± SEM (n = 3).

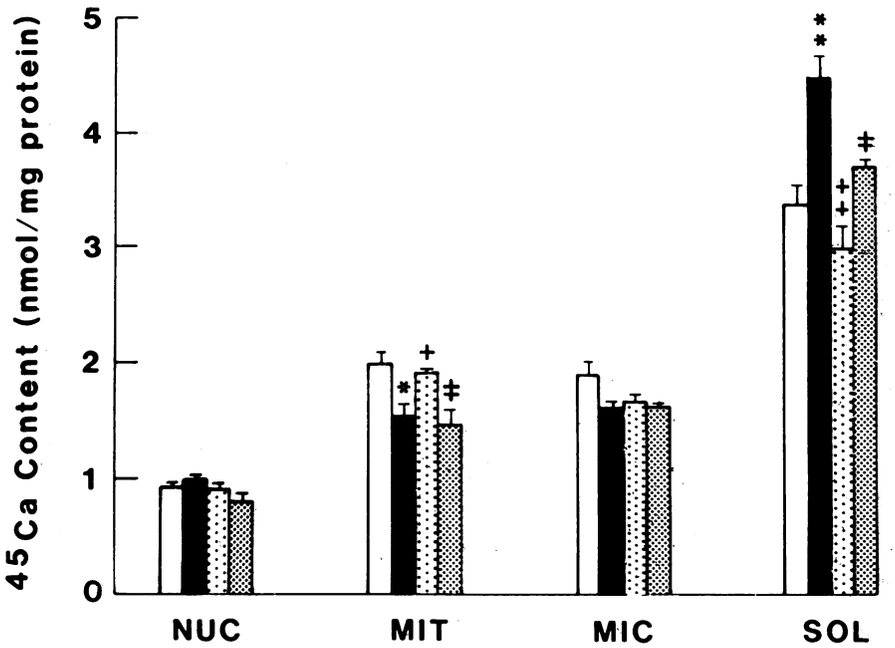

FIG. 6. CHF_2-Orn suppresses the isoproterenol-induced changes in subcellular distribution of ^{45}Ca in kidney cortex, and putrescine reverses CHF_2-Orn suppression. Cortex slices were preincubated in medium containing ^{45}Ca (3–4 μCi/ml) for 20 min at 37°C; 5 mM CHF_2-Orn, CHF_2-Orn and 0.5 mM putrescine, or no agent was then added and the preincubation was ended 10 min later. Slices were rinsed, incubated in fresh medium with 1 μM isoproterenol (■), isoproterenol and CHF_2-Orn (▨), isoproterenol, CHF_2-Orn, and putrescine (▩), or no additions (□, control) at 37°C for 10 min, homogenized, and fractionated by differential centrifugation into a nuclear (NUC), mitochondrial (MIT), microsomal (MIC), and soluble fraction (SOL) (12, 14). Data are means ± SEM (n = 3).

intracellular message for the generation of a Ca^{2+} signal involves a receptor-mediated, Ca^{2+}-dependent activation of a latent OrnDCase located in or near the plasmalemma. Message transmission is effected by polyamines synthesized initially in the subplasmalemmal microdomain. Signal reception involves a cation-exchange reaction, whereby polyamines bind to anionic sites in the plasmalemma, mitochondria, and other cytomembrane systems and release bound calcium as free cytosolic Ca^{2+}. We postulate that calcium gating involves a change in conformation of calcium channel molecules resulting from a polyamine-mediated displacement of bound calcium. This inference is consistent with the model that calcium gate closure results from a binding of Ca^{2+} to calcium channel molecules at the inner membrane surface (21).

We recently showed that testosterone induces an acute (<30 sec) short-lived increase in OrnDCase activity and an early (<2 min) sustained increase in polyamines in mouse kidney cortex; this polyamine synthesis appears to be required for the stimulation of Ca^{2+} fluxes and the accompanying increase in endocytosis, hexose transport, and amino acid transport (13, 14). In addition, a number of surface agonists, including phorbol myristate acetate, concanavalin A, and the prostaglandins PGA_2 and PGE_2, have been found to evoke a rapid increase in OrnDCase and polyamine levels in mouse kidney cortex (20) and concurrently induce a Ca^{2+}-dependent stimulation of membrane transport functions (10). These findings support the hypothesis that OrnDCase activation and polyamine synthesis play a pivotal role in signal transduction and transmission and stimulus–response coupling in cells. Important issues to be resolved by future work include an elucidation of the molecular mechanism of the rapid activation–inactivation cycle of OrnDCase and an experimental assessment of the polyamine–Ca^{2+} exchange reaction hypothesis for the control of calcium gating and the mobilization of intracellular calcium.

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