The Role of Calcium in Follicle-stimulating Hormone Signal Transduction in Sertoli Cells*

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Sertoli cells are hormonally regulated by follicle-stimulating hormone (FSH) acting upon a G-protein-linked cell surface FSH receptor. FSH increases intracellular cyclic AMP but the involvement of other signal transduction mechanisms including intracellular calcium in FSH action are not proven. Using freshly isolated rat Sertoli cells we measured cytosolic free ionized calcium levels by dual-wavelength fluorescence spectrophotometry using the calcium-sensitive fluorescent dye Fura2-AM. The cytosolic calcium concentration in unstimulated Sertoli cells was 89 ± 2 nM (n = 151 experiments) and was markedly increased by either calcium channel ionophores (ionomycin, Bay K8644) or plasma membrane depolarization consistent with the presence of voltage-sensitive and -independent calcium channels in Sertoli cell membranes. Ovine FSH stimulated a specific, sensitive (ED50, 5.0 ng of S-16/ml), and dose-dependent (maximal at 20 ng/ml) rise in cytosolic calcium commencing within 60 s to reach levels of 192 ± 31 nM after 180 s and lasting for at least 10 min. The effect of FSH was replicated by forskokinin, chola toxin, and dibutyryl cyclic AMP, suggesting that cyclic AMP may mediate the FSH-induced rise in cytosolic calcium. The FSH-induced rise in cytosolic calcium required extracellular calcium and was abolished by calcium channel blockers specific for dihydropyridine (verapamil, nicardipine), nonvoltage-gated (rutheinum red) or all calcium channels (cobalt). Thus FSH action on Sertoli cells involves a specific, rapid, and sustained increase in cytosolic calcium which requires extracellular calcium and involves both dihydropyridine-sensitive, voltage-gated calcium channels and voltage-independent, receptor-gated calcium channels in the plasma membranes of rat Sertoli cells. The replication by cyclic AMP of the effects of FSH suggests that calcium may be a signal-amplication or -modulating mechanism rather than an alternate primary signal transduction system for FSH in Sertoli cells.

Sertoli cells are primarily under the hormonal regulation of FSH while fulfilling numerous functions geared toward supporting the development and maturation of germinal cells. FSH acts upon plasma membrane receptors located on the basolateral surface of Sertoli cells contiguous with the extraluminal or basal compartment of the seminiferous tubules. Previous studies have suggested a role for cyclic AMP as a second messenger mediating FSH action in Sertoli cells. However, evidence for the involvement of other signal transduction systems for FSH action in Sertoli cells has only recently emerged.

Although previous investigations have implicated the intracellular calcium in mediating dynamic changes in the cytoskeleton (2) or FSH signal transduction mechanisms (3-5), neither basal cytosolic calcium levels nor the effects of FSH stimulation on cytosolic calcium concentrations in Sertoli cells have been reported. We have therefore studied the cytosolic calcium level in resting and FSH-stimulated immature rat Sertoli cells in vitro. After finding a prominent rise in cytosolic calcium induced by FSH stimulation, we investigated the nature of the calcium channels involved, the source of calcium, as well as the relationship of the rise in cytosolic calcium to the generation of cyclic AMP as a second messenger for FSH.

**EXPERIMENTAL PROCEDURES**

**Solutions and Reagents**—The standard saline solution for spectrofluorimetry consisted of 145 mM sodium chloride, 5 mM potassium chloride, 2 mM calcium chloride, 1 mM magnesium sulphate, 10 mM glucose buffered by 10 mM HEPES and titrated to pH 7.4 with sodium hydroxide. Calcium-free saline was prepared similarly except replacing 2 mM calcium chloride with 2 mM disodium EGTA. Depolarizing solutions analogous to standard and calcium-free saline were prepared identically apart from exchanging sodium and potassium ion concentrations. Calibration solution for minimal fluorescence (low calcium) consisted of 145 mM sodium chloride, 5 mM potassium chloride, 1 mM magnesium sulphate, 15 mM dipotassium EGTA, 10 mM glucose, 10 mM HEPES, 30 mM TAPS buffer at pH 8.3. Calibration solution for maximal fluorescence (high calcium) consisted of standard saline with added 10 mM calcium chloride 0.1% Triton X-100. Bay K8644 and ionomycin were purchased from Calbiochem. Ovine FSH, human chorionic gonadotropin, rat growth hormone, and prolactin were obtained from National Institute of Arthritis, Metabolism, and Digestive Diseases. All other chemicals were of analytical grade and obtained from Sigma. Fura-2AM, ionomycin, Bay K8644, nicardipine, and forskolin were diluted in dimethyl sulfoxide with the

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1 The abbreviations used are: FSH, follicle stimulating hormone; Fura-2AM, [1-[2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-ox]-2-(2′-amino-5′-methylphenoxy)-ethane-N,N,N′,N′-tetraacetic acid, pentaacetoxymethyl ester]; ACTH, adrenocorticotropic hormone; HEPES, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid; EGTA, [ethylene (oxymetheninitriolo)]tetraacetic acid; TAPS, 3-[N,N-dimethylaminomethyl]-2-bis(hydroxymethyl)propanesulfonic acid; Bay K 8644, 1,4-dihydropyridine-5-nitro-4-[3-(tri-fluoromethyl)phenyl]-3-pyridine carboxylic acid methyl ester; LH, luteinizing hormone.
Sertoli cells exposed to a final concentration of dimethyl sulfoxide of less than 0.2%.

Isolation of Sertoli Cells—Tests were excised rapidly from five 17- to 20-day-old Wistar rats, placed in Hanks' balanced salt solution containing Dnase (10 mg/liter), and digested in a single-stage digestion method developed by Dr. J. A. Grootepe (Erasmus University, Rotterdam, The Netherlands). Decapsulated testes were digested in 10 ml of Hanks' balanced salt solution containing 10 mg/liter of Dnase, 1 g/liter collagenase, 1 g/liter hyaluronidase and 1 g/liter trypsin in a shaking water bath (37 °C, 120 cycles/min, 30 min). Sertoli cells were then washed and centrifuged (22 °C, 2 min, 25 × g) three times in 50 ml of Hanks' balanced salt solution containing 10 mg/liter of Dnase. This preparation contains 97 ± 1% Sertoli cells identified morphologically by light microscopy (n = 6 digests, 2241 total cells counted) after prolonged digestion to obtain single cells.

Measurement of Cytosolic Calcium in Sertoli Cells—Preliminary experiments using fluorescent microscopy indicated that the optimal loading conditions for Fura into Sertoli cells was an incubation for 45 min at 37 °C with 20 μM Fura-2 AM (stock 10 mg/ml in dimethyl sulfoxide, final dilution 1:500) dissolved in Eagles' minimal essential medium, containing 1 g/liter bovine serum albumin in an atmosphere of 5% carbon dioxide in air. After loading incubation, Sertoli cells were washed and centrifuged (22 °C, 2 min, 25 × g) three times in standard saline solution and finally resuspended in 25 ml of the appropriate solution. For spectrofluorometry, a 1.5-ml solution of Sertoli cells was placed into a water-jacketed quartz cuvette maintained at 37 °C by recirculating warm water maintained thermostatically throughout all experiments. Sertoli cells in the cuvette were kept suspended by mixing with a small magnetic stirrer and all recordings were completed within 2 h of loading with Fura.

Emission fluorescence for calculation of cytosolic calcium was routinely recorded for 60 s prior to and for 180 s after administration of ovine FSH and/or other drugs (or saline vehicle) in 30 μl of cell incubation medium. In order to standardize cytosolic calcium measurements, cytosolic calcium levels were routinely estimated at 180 s after administration of stimuli (or 240 s after the start of the experiment) unless otherwise indicated. In the experiments involving calcium channels blockers, the Sertoli cells were preincubated with drug in the standard saline solution for 20 min at room temperature (22 °C) prior to fluorescence measurements. In the studies undertaken in calcium-free saline and depolarizing saline, cells were transferred from standard saline to the appropriate saline (calcium-free or depolarizing) immediately prior to fluorescence measurement.

Fluorescence was recorded in a spectrofluorometer (Hitachi F-4010, Tokyo) equipped with dual-excitation wavelength with a minimum recording time of 4 s for Fura. Alternating excitation wavelengths at 340 nm and 380 nm and emission wavelength at 510 nm were employed and data was recorded on a diskette. Cytosolic calcium was subsequently calculated from the recorded fluorescence intensity using software provided by the manufacturer implementing the method of Grynkiewicz et al. (6).

Free calcium (nM) = Kd × (R - Rmin/Rmax - R) × Sf2/Sb2

with Kd being the Fura dissociation constant at 37 °C (224 nM); R defined as the ratio of fluorescence measured at 340- and 380-nm excitations, respectively; and Sf2/Sb2 the ratio of fluorescence at 380 nm in low calcium to that of high calcium. The calibration procedure to estimate Rmin, Rmax, and autofluorescence was performed for every experiment individually as discussed elsewhere (7, 8).

Data Analysis—Each experiment was undertaken in at least four independent replicate isolations of Sertoli cells. All results are expressed as the mean ± S.E. of individual observations. Statistical analysis was performed by analysis of variance for repeated measures and unpaired t test as appropriate.

RESULTS

Cytosolic Calcium Concentrations under Basal Conditions and after FSH Stimulation in Sertoli Cells—Sertoli cell cytosolic calcium levels under basal conditions (averaged over 60 s) were 89 ± 2 nM (n = 151 experiments). Administration of ovine FSH (1 mg/liter) induced a significant rise in cytosolic calcium consistently within 60 s (Fig. 1) and which was sustained for at least 10 min (data not shown). All subsequent

\[ R^{\text{calc}} = R^{\text{basal}} + \Delta R^{\text{calc}} = R^{\text{basal}} + K_d \times \left( \frac{R_{380} - R_{340}}{R_{380} - R_{420}} \right) \]

with \( R^{\text{basal}} \) being the fluorescence ratio at 380 and 340 nm in the absence of calcium, \( R^{\text{calc}} \) the fluorescence ratio at 380 and 340 nm in the presence of calcium, and \( K_d \) the dissociation constant of the Fura-2 calcium sensor.

\[ \Delta R^{\text{calc}} = R^{\text{calc}} - R^{\text{basal}} \]

The FSH-induced rise in cytosolic calcium in Sertoli cells was highly specific since other pituitary and related hormones (human chorionic gonadotropin, 1 ng/liter, 87 ± 14 nM, n = 4; adrenocorticotropic (1 ng/liter, 91 ± 13 nM, n = 5); rat prolactin (1 ng/liter, 85 ± 13 nM, n = 4) and rat growth hormone (1 ng/liter, 85 ± 25 nM, n = 4) had no effect on cytosolic calcium compared with saline-treated Sertoli cells (87 ± 13 nM, n = 7).

Effects of Calcium Channel Ionophores, Ionomycin, and Bay K8644 on Cytosolic Calcium in Sertoli Cells—Ionomycin (100 nM), a nonelective ionophore, induced a dramatic rise in cytosolic calcium peaking at 934 ± 281 nM (n = 5) between 20 and 40 s after drug administration with levels remaining markedly elevated (568 ± 69 nM) at 180 s (Fig. 3). Bay K8644 (1000 nM), a selective dihydropyridine agonist, also induced a brisk but smaller rise in cytosolic calcium to 247 ± 86 nM (n = 5) within 20 s but which remained elevated (239 ± 36 nM) at 180 s following drug administration (Fig. 3).

Dose Response of FSH Effect on Cytosolic Calcium in Sertoli Cells—Sertoli cells exposed to increasing doses of ovine FSH demonstrated a dose-dependent increase in cytosolic calcium concentration (Fig. 4). Cytosolic calcium increased within 20–60 s and remained elevated for 180 s after FSH administration. The median effective FSH dose was approximately 5 ng/ml and a dose of 20 ng/ml had essentially maximal effects.

FIG. 1. Cytosolic calcium levels in rat Sertoli cells measured by dual-wavelength excitation at 4- s intervals under baseline conditions before and after administration of either FSH (1 ng/liter) or saline vehicle control. Symbols indicate mean ± S.E. of replicates of experiments, and numbers of experiments are indicated in parentheses.

FIG. 2. Cytosolic calcium levels in rat Sertoli cells measured by dual-wavelength excitation at 20-s intervals before and after administration of either FSH (1 ng/liter) or saline vehicle control. Symbols indicate mean ± S.E. of replicates of experiments, and numbers of experiments are indicated in parentheses.

\[ \Delta [Ca^{2+}] = \frac{F_{380} - F_{340}}{F_{380} - F_{420}} \times \frac{[Ca^{2+}]_{\text{sat}} - [Ca^{2+}]_{\text{free}}}{[Ca^{2+}]_{\text{free}}} \]

with \( F_{380} \) and \( F_{340} \) being the fluorescence at 380 and 340 nm, respectively, \( [Ca^{2+}]_{\text{free}} \) the free calcium concentration, and \( [Ca^{2+}]_{\text{sat}} \) the saturation calcium concentration.
to raise intracellular cAMP concentration by a variety of means not involving the FSH receptor. The agents used were dibutyryl cAMP (a membrane-permeable analog of cyclic AMP), forskolin, and cholera toxin (direct activators of adenylate cyclase).

All three agents which raise intracellular cAMP caused a significant increase in cytosolic calcium levels although their time-courses were different (Fig. 5). Forskolin (50 μM) produced a rapid rise in cytosolic calcium with near-maximal levels achieved within 20 s and rising to 186 ± 26 nM (n = 10) at 180 s after administration. Cholera toxin (10 ng/liter) generated a slower response with a significant increase only at 120 s but rising to 152 ± 52 nM (n = 5) at 180 s after administration. Dibutyryl cAMP (1 mM) had an intermediate effect with calcium levels rising gradually after 60 s of administration to a concentration of 149 ± 25 nM (n = 10). In comparison, after injection of vehicle saline to unstimulated Sertoli cells the cytosolic calcium levels remained at 87 ± 13 nM (n = 7).

**Effects of Calcium Channel Blockers on Resting and FSH-stimulated Cytosolic Calcium Levels in Sertoli Cells**—This set of experiments was designed to utilize blockers of either (i) voltage-gated (nicardipine, verapamil, diltiazem) or (ii) non-voltage-gated calcium channels (ruthenium red) or (iii) non-specific blocker of all calcium channels (cobalt chloride).

In the presence of 2 mM extracellular calcium, all the calcium channel blockers, except diltiazem, reduced both the resting cytosolic calcium concentrations and abolished the FSH-induced rise in cytosolic calcium (Table, I, Fig. 6). In contrast, however, diltiazem did not affect either the basal or the FSH-induced increase in cytosolic calcium concentration.

**Effects of Calcium-free Medium on Basal and FSH-stimulated Cytosolic Calcium Levels in Sertoli Cells**—The dependence of the FSH-induced rise in cytosolic calcium on extracellular calcium was determined by replacing the bathing medium with calcium-free medium containing EGTA. Under these conditions the basal cytosolic calcium was reduced to 36 ± 5 nM (n = 6) and following FSH administration the cytosolic calcium did not increase 42 ± 4 nM (n = 10) (Fig. 7).

**Effects of Reversal of Sodium and Potassium Concentrations in Medium on Basal and FSH-stimulated Cytosolic Calcium Levels in Sertoli Cells**—Exchanging sodium and potassium concentrations in the extracellular medium (high potassium, low sodium) led to an immediate increase in cytosolic calcium to 143 ± 23 nM (n = 9) and subsequent FSH administration did not further increase these levels [169 pm 27 nM (n = 7)] (Fig. 8). The absence of external calcium in the incubation medium abolished both the initial rise in cytosolic-free calcium concentration as well as the FSH-induced increase [86 ± 23 nM (n = 8) vs. 75 ± 29 nM (n = 6)].

**DISCUSSION**

The present findings provide the first direct measurement of cytosolic-free calcium in rat Sertoli cells and demonstrate that physiological doses of FSH, the major trophic hormone for Sertoli cells, stimulate a prompt rise in cytosolic calcium. This provides a direct basis for previous suggestions (2, 3) that calcium might be involved in the FSH signal transduction mechanism.

**Table I**

| Treatment     | Dose | Cytosolic calcium concentrations |
|---------------|------|---------------------------------|
|               | μM   | nM    | Stimulated* |
|               | Base line* | FSH  | Saline      |
| Saline        | Control | 79 ± 6 | 192 ± 13 | 103 ± 7 |
| Viscapamil    | 100   | 49 ± 5 | 69 ± 6  | 69 ± 3  |
| Nicardipine   | 50    | 45 ± 6 | 21 ± 4  | 20 ± 9  |
| Diltiazem     | 100   | 82 ± 4 | 173 ± 15 | 101 ± 11|
| Ruthenium red | 100   | 67 ± 5 | 71 ± 6  | 62 ± 11 |
| Cobalt chloride | 100   | 64 ± 5 | 48 ± 10 | 49 ± 8  |

* Mean cytosolic calcium concentrations averaged over the 60 s before administration of FSH or saline vehicle control.

* Mean cytosolic calcium concentrations at 180 s after administration of FSH (1 ng/liter) or saline vehicle control.
We used a modified cell isolation technique to provide a more rapid and higher yield of Sertoli cells and measured cytosolic calcium by dual-wavelength fluorometry using the intracellular fluorescent dye Fura-2AM. Using these methods, the cytosolic-free ionized calcium concentration was approximately 90 nM in unstimulated rat Sertoli cells in the presence of 2 mM extracellular calcium. FSH induced a rapid and reproducible rise in cytosolic calcium with clear dose and time dependence. The median effective dose of ovine FSH was about 5 ng/ml and a maximally effective dose (>10 ng/ml) increased cytosolic calcium by about 100 nM consistent with a physiological effect. The rise in cytosolic calcium was both rapid and sustained being evident within 60 s and lasting undiminished for at least 10 min. The effect of FSH was highly specific as indicated by the failure of supraphysiological doses of related pituitary hormones (growth hormone, prolactin, ACTH) or human chorionic gonadotropin (which acts upon LH receptors) to modify cytosolic calcium concentration. These results are also consistent with recent findings that FSH stimulates increases in cytosolic calcium in the rat granulosa cell, the ovarian homolog of the Sertoli cell (9).

It is well established that interaction of FSH with its plasma membrane receptor on Sertoli cells generates an increase in intracellular cAMP (2). This rise in intracellular cAMP has long been believed to be the sole or principal FSH signal transduction system in Sertoli cells (2). The present study indicates that the activation of calcium flux through one or more types of calcium channels is however intimately involved in biochemical expression of FSH action. Whether FSH-induced calcium fluxes are an alternate primary signal transduction mechanism or a signal amplification or modulating mechanism in Sertoli cells remains unclear. The full replication of FSH-induced rise in cytosolic calcium by both a membrane permeable cyclic AMP analog (dbcAMP) as well as agents which increase intracellular cyclic AMP by nonreceptor-mediated activation of adenylylate cyclase (forskolin and cholera toxin) favors the possibility that cytosolic calcium fluxes may represent a mechanism of signal amplification or modulation rather than a primary signal. Since the FSH receptor contains a classical G-protein-linked domain (10, 11), activation of the FSH receptor could induce calcium channel activation by phosphorylation (and thereby activation) of calcium channel proteins through the activity of the catalytic subunit of cAMP-dependent protein kinase A (12). The exact nature of the linkage between these two signals remains, however, to be further clarified.

The present data indicate that FSH is one of a variety of hormones which utilize increases in cytosolic calcium as a signal transduction system (13). The involvement of calcium in FSH signal transduction is analogous to its role in LH signal transduction in which both cAMP and calcium fluxes are stimulated (14, 15). LH and FSH are closely related, dimeric pituitary glycoprotein hormones sharing an identical subunit, highly homologous β subunits (16), and cell surface receptors which are members of the G-protein-linked membrane receptor family (10, 17). Both the hormones and their receptors are thought to derive from common ancestral genes (10). Thus it is likely that their signal transduction mechanisms may have preserved common features. The LH receptor signal transduction mechanisms do involve calcium fluxes (14, 15) and recent evidence suggests that calcium may be an alternate primary signalling mechanism rather than just an amplification signal (15). This evolutionary evidence supports the possibility that FSH receptor signalling may involve calcium fluxes as a primary and/or amplification/modulating signal.
The abolition of the FSH-induced rise of cytosolic calcium by the removal of extracellular calcium and by blockers of both voltage-gated and voltage-independent calcium channels indicates that more than one class of calcium channels is involved in the FSH-induced rise of cytosolic calcium. It also extends previous findings using radioactive calcium tracer where the presence of both voltage-gated and nonvoltage-gated calcium channels in the plasma membrane of Sertoli cell had been inferred (3). This concept is further supported by the finding that a nonselective ionophore (ionomycin) had far greater effects on cytosolic calcium than an ionophore selective for voltage-gated channels (Bay K8644). Furthermore, calcium channel blockers selective for voltage-gated channels (nicardipine, verapamil) as well as for voltage-independent channels (rubidium red) lowered the basal cytosolic calcium levels and prevented the FSH-induced rise.

Despite the consistent observations on the involvement of voltage-gated calcium channels, surprisingly diltiazem, a benzothiazepine blocker of voltage-gated calcium channels, did not alter the FSH-induced rise in cytosolic calcium at doses exceeding those effective in other cells (18-20). The selective failure of diltiazem to block FSH-induced rise of cytosolic calcium in rat Sertoli cells is unexpected because all three classes of calcium channel blockers bind to the same a-1 subunit of the calcium channel proteins (18, 21). Since prototypes of two of the three classes are effective, it is unlikely that a major structural variant of the calcium channel molecule is involved. Similarly, as diltiazem does block calcium channels in other rat tissues (22, 23), a species-specific variant in calcium channel structure is also not a plausible explanation. A more likely explanation is heterogeneity in tissue sensitivity within a species to diltiazem in blocking voltage-gated calcium channels as previously reported (19, 24). Electrophysiological and radioligand binding studies reveal the existence of tissue-specific isoforms of L-type voltage-dependent calcium channel (25-27). This class of channels comprises a group of similar yet distinct subunits of protein complexes that differ in kinetics and voltage dependence (28, 29) as well as in primary structure (21, 27, 30) in various cells of the same organism. Thus the lack of response to diltiazem may be attributable to tissue-specific variants in the a-1 subunit of the L-type calcium channel protein. The detailed structure of the calcium channel proteins expressed and active in Sertoli cell membranes, however, needs to be characterized to clarify this problem further.

The importance of voltage-gated channels is reinforced by the observation that exposure of the Sertoli cells to an extracellular environment of reversing potassium and sodium ion concentrations (high potassium, low sodium) caused an elevation in cytosolic-free calcium similar to that induced by FSH or cyclic AMP. This suggests that depolarizing the Sertoli cell plasma membrane activates the voltage-sensitive calcium channels. Since Sertoli cells are not excitable and are exposed, in situ, to extracellular fluids with potassium concentrations ranging from 5 to 50 mM (31), the electrophysiological characteristics of this cell are still poorly defined. FSH has been shown to induce hyperpolarization of Sertoli cell plasma membranes (5, 32, 33), followed by a depolarization (34) which might trigger the activity of voltage-gated calcium channels in addition to any potential effects of cyclic AMP.

The lowering of basal cytosolic calcium and blockade of the FSH-induced increase in cytosolic calcium by rubidium red and cobalt as well as the prominent effect of ionomycin to increase cytosolic calcium indicates that non-voltage-gated calcium channels are important in FSH signal transduction systems in Sertoli cells. The decrease of resting cytosolic calcium and blockade of the FSH-induced rise when Sertoli cells are incubated in calcium-free medium is also consistent with the requirement for external calcium in resting conditions as well as in FSH signal transduction mechanisms. Since, however, FSH binding to its receptor is partially dependent upon calcium (35, 36), it is possible that the blockade of the FSH-induced rise is at least partly due to blockade of FSH binding to its receptor.

An important question is whether the source of calcium which is involved in the FSH-induced flux is external or internal in the Sertoli cell. Previous data utilizing radioactive calcium has demonstrated that transmembrane calcium flux from extracellular calcium sources is activated by FSH (3, 4). The requirement for external calcium and the involvement of plasma membrane calcium channels indicated by ionophore and blocker experiments in the rise of cytosolic calcium due to FSH argue that an external calcium source is involved. In contrast, voltage-independent, receptor-mediated calcium flux from intracellular sources could be invoked by the generation of inositol triphosphate, an endogenous ionophore. The sustained elevation of cytosolic calcium observed in our studies, however, is not consistent with the fast transient rise in cytosolic calcium observed when IP3 generation is stimulated by a receptor-mediated mechanism (37, 38). Furthermore, FSH has been shown to inhibit rather than stimulate phosphoinositide turnover in Sertoli cells (39, 40). Thus, an external source is most consistent with current knowledge, however, further studies would be required to exclude definitively the possibility of calcium mobilization from intracellular stores in the Sertoli cell.

In conclusion, the present study demonstrates the involvement of calcium flux in the FSH signal transduction mechanisms in Sertoli cells. It is most likely that multiple types of plasma membrane calcium channels are involved in the receptor-initiated events leading to a cyclic AMP-mediated influx of calcium from extracellular sources. The underlying mechanisms by which FSH receptor is coupled with calcium channels of plasma membrane of Sertoli cells is still not resolved and constitutes a major challenge for further studies.

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