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Inhibition of Ornithine Decarboxylase Activity by Follicle Stimulating Hormone in Primary Culture of Rat Sertoli Cells

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The effect of follicle stimulating hormone on the activity of ornithine decarboxylase (ODC) was determined in primary culture of rat Sertoli cells. Three different FSH preparations (NIH oFSH-S-15, S-16, and eFSH) inhibited ODC activity in rat Sertoli cells under different media conditions. The inhibition was both time- and dose-dependent. The mechanism of the FSH inhibitory effect was studied using dibutyryl cyclic adenosine monophosphate (dbcAMP), 1-methyl-3-isobutylxanthine (MIX), forskolin, and isoproterenol. All of these agents, known to elevate cellular cAMP levels, inhibited ODC activity in cultured rat Sertoli cells. The combined effect of each of these substances plus FSH was either greater than, or equal to, that of FSH alone, and was not additive. Dibutyryl cyclic guanosine monophosphate had no effect on the ODC activity. These findings suggest that FSH inhibition of ODC activity in the rat Sertoli cell may be mediated by cAMP.

Key words: ornithine decarboxylase, follicle stimulating hormone, rat Sertoli cells.

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Ornithine decarboxylase (L-ornithine carboxylyase, EC 4.1.1.17) (ODC) is the rate-limiting enzyme in the polyamine biosynthetic pathway. The activity of ODC is elevated under conditions of rapid cell growth (Jänne et al, 1978; Fozard et al, 1980; Heby, 1981) and in target organs in response to stimulation by either polypeptide or steroid hormones (Kobayashi et al, 1971; Levine et al, 1973; Russell et al, 1976; Russell, 1985). Studies *in vivo*, using follicle stimulating hormone (FSH), demonstrated stimulation of rat testicular ODC activity and increased production of polyamines (MacIndoe and Turkington, 1973; Reddy and Villee, 1975).

The Sertoli cells, which are nongerminal components of the seminiferous tubule, are thought to play a supportive role in germ cell development. They have been shown to contain nearly all of the ODC activity of the testis (MacIndoe and Turkington, 1973). Sertoli cells are mainly regulated by FSH, and ODC activity in bovine Sertoli cells has been reported by Francis et al (1981) to be induced by FSH. However, they did not observe FSH stimulation of ODC activity in rat Sertoli cells.

In this study, we investigated the effect of FSH on

ODC activity in Sertoli cells isolated from 18-day-old rats and cultured in different media. We also explored the effect of dibutyryl cAMP, 1-methyl-3-isobutylxanthine, forskolin and isoproterenol on ODC activity in Sertoli cells in an attempt to elucidate the mechanism of ODC inhibition by FSH.

Materials and Methods

Materials

Ovine FSH (NIH oFSH-S-15 and NIH-oFSH-S-16) was obtained from the NIADDK, National Hormone and Pituitary Program, and highly purified equine FSH was a generous gift from Dr. Darrell N. Ward (University of Texas System Cancer Center at Houston). The eFSH has an activity of 100 × NIH FSH S-13 in the chicken testis FSH radioreceptor assay (Bousfield and Ward, 1984). Ornithine, dithiothreitol, pyridoxal phosphate, dibutyryl cAMP (dbcAMP), dibutyryl cGMP (dbcGMP), 1-methyl,3-isobutylxanthine (MIX), forskolin, and isoproterenol were obtained from Sigma Chemical Co. (St. Louis, MO). [1-^{14}C]L-ornithine hydrochloride (57 mCi/mmol) was purchased from Radiochemical Centre, Amersham (Arlington Heights, IL). Powdered media were obtained from Flow Biochemicals (Rockville, MD). Immature (18-day-old) male Sprague-Dawley rats were supplied by Timco (Houston, TX).

Sertoli Cell Culture

Rat Sertoli cells were isolated by the method of Steinberger et al (1975) and cultured at 34 C in DFM (equal parts of Dulbecco Modified Eagle's medium and Ham's F-10). The cells were maintained in 100-mm Corning culture dishes containing 5 ml medium under 5% CO_2-95% air atmosphere until they spread to form a nearly continuous monolayer. After 3 days in culture, the medium was changed to either DFM, DFM-6F (DFM supplemented with insulin, 1.0 μg/ml; epidermal growth factor, 10 ng/ml; Vitamins A and E, 200 ng/ml; progesterone and hydrocortisone, 10^{-8}M) or DFM-7F (DFM-6F plus transferrin, 5 μg/ml). After 2 days, the culture media were replaced again with DFM, DFM-6F or DFM-7F, respectively, containing FSH or other agents. After the required time intervals, the media were removed, the cells were rinsed twice with Tris-saline (10 mM Tris-HCl, pH 7.4 at 4 C, 0.85% NaCl), harvested in 1.0 ml of buffer (25 mM Tris-HCl, pH 7.4, 0.1 mM EDTA, 1.0 mM DTT) per dish and frozen at −70 C. Immediately prior to ODC assay, the cells were thawed, sonicated for 30 seconds at 4 C and centrifuged at 25,000 × g for 30 minutes. The supernatants were assayed in duplicate for ODC activity.

TABLE 1. Time Course of FSH Action on Sertoli Cell Ornithine Decarboxylase Activity*

Media	Time (h)	ODC Activity (pmoles/h/mg protein) Control	FSH	Significance
DFM	2	237 ± 35	210 ± 13	N.S.
	4	1329 ± 86	758 ± 65	$P < 0.01$
	8	932 ± 27	476 ± 59	$P < 0.01$
	24	315 ± 71	217 ± 30	N.S.
DFM-6F	4	3481 ± 172	1829 ± 1	$P < 0.05$
	6	3570 ± 444	1262 ± 7	$P < 0.01$
	8	3612 ± 807	1921 ± 42	$P < 0.05$
DFM-7F	4	4450 ± 106	2427 ± 146	$P < 0.01$
	8	3069 ± 47	1339 ± 111	$P < 0.01$
	12	2111 ± 184	1823 ± 10	N.S.

*Rat Sertoli cells were treated with FSH (oFSH-S-15, 5 μg/ml) for specified periods of time in three different media conditions. ODC activity was determined by $^{14}CO_2$ released from cell homogenates. Data represent mean ± SE (n = 4) and were analyzed by one-way Anova and Duncan's multiple range test. The comparisons were made within the same group between FSH-treated and untreated controls.

Biochemical Determination of ODC Activity

The ODC activity in the cell preparations was determined as described by Jänne and Williams-Ashman (1971) with some modifications (Madhubala and Reddy, 1980). Briefly, ODC activity was determined by the amount of $^{14}CO_2$ released from [1-^{14}C]L-ornithine in a 1-h assay at 37 C. The reaction mixture contained 100 mM Tris-HC1, pH 7.4; 1 mM L-ornithine; 0.4 μCi [1-^{14}C]L-ornithine; 0.2 mM pyridoxal phosphate; 5 mM dithiothreitol; and 240 μl of the enzyme in a final volume of 500 μl. Duplicates of the blank control were always performed in parallel by omitting Sertoli cell cytosol from the reaction mixtures. Protein content of the supernatant was estimated according to the method of Lowry et al (1951). ODC activity is expressed as pmoles or nmoles of $^{14}CO_2$ liberated per hour per mg protein. Results were analysed by one way Anova and Duncan's multiple range test.

Results

ODC Activity in Sertoli Cells

Sertoli cells responded to fresh media change by an increase in their ODC activity (Fig. 1 and Table 1).

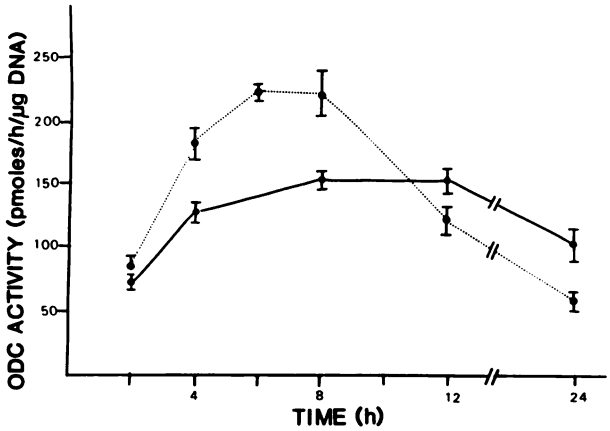

Fig. 1. Effect of fresh media on the basal ODC activity of cultured rat Sertoli cells. The solid line (——) represents the effect of fresh DFM-6F and the dotted line (·····) that of DFM-7F on ODC activity. The data (mean ± SE; n = 4) were analyzed by one-way Anova and Duncan's multiple range test.

Sertoli cells exhibited an overall lower ODC activity when they were cultured in DFM as compared with those cells in 6F- or 7F-supplemented media. In general, the ODC activity reached a peak at 4 hours after media change. In DFM media, Sertoli cell ODC activity reached maximum at 4 hours, declined by 8 hours and declined further by 24 hours. In the presence of 6F and 7F, the Sertoli cells also showed high ODC activity at 4 hours, remained high from 4 hours up to 12 hours (varied from culture to culture), and then declined by 24 hour post-media change.

Effect of FSH on Sertoli Cell ODC Activity at Various Time Intervals

FSH-S-15 (5 μg/ml) was added to Sertoli cell cultures in three different media. In all media, ODC activity of the FSH-treated cells markedly decreased (30 to 60% of control) between 4 to 8 hours after treatment (Table 1) as compared with non-treated controls. Activity returned to near control levels at 24 hours. It appears that the maximum decline (33% of control) occurred at 6 hour posttreatment.

Dose-Dependent Inhibition of ODC Activity by FSH

Figure 2 shows the effect of various doses (0.01 to 5.0 μg/ml) of FSH on ODC activity after 6 hours of treatment. A slight decrease in Sertoli cell ODC activity occurred in the presence of 0.01 μg/ml FSH. At 0.01 μg/ml to 0.1 μg/ml of FSH, the inhibitory effect of FSH appeared to be linear. Although 0.1 μg/ml of FSH was sufficient to elicit maximum inhibition, we elected to use 0.5 μg/ml of FSH in subsequent studies to ensure the effect, since this dose showed a similar degree of suppression. Highly purified eFSH (5 ng/ml and 10 ng/ml) showed inhibition of ODC activity (Table 2) similar to that observed with NIH oFSH-S-15 (Table 1) and NIH oFSH-S-16.

Effect of dbcAMP, MIX, Forskolin and Isoproterenol on ODC Activity in Rat Sertoli Cells

As shown in Table 3, dbcAMP (2.5 mM) inhibited ODC activity dramatically when measured 4 hours after its addition to the cultures. This inhibition was specific for cAMP as Na-butyrate had no effect on ODC activity. The combined treatment with FSH and dbcAMP showed a decrease in ODC activity that was equal to or greater than that resulting from either substance alone; however, the effect was not additive.

The inclusion of MIX (0.1 μg/ml), forskolin (5 μM) or isoproterenol (5 μM) in the media also inhibited Sertoli cell ODC activity. Combined treatment with FSH plus either MIX, forskolin, or isoproterenol showed a decrease in ODC activity. The combined effect was greater than, or equal to, that of either substance alone, but was not additive.

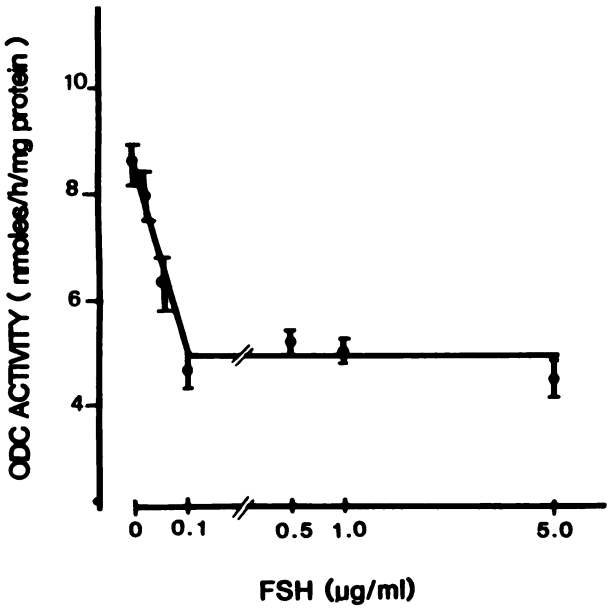

Fig. 2. Effect of different doses of FSH (oFSH-S-15) on ornithine decarboxylase activity of rat Sertoli cells after 6 hours of treatment in DFM-6F. Enzyme activity was determined as mentioned in materials and methods section. Each point represents mean ± SE (n = 4) and was analyzed by one-way Anova and Duncan's multiple range test.

Effect of dbcGMP on ODC Activity

The effect of dbcGMP (2.5 mM) on ODC activity and the combined effect of dbcGMP and FSH is shown in Table 3. The dbcGMP had no effect on ODC activity in Sertoli cells. The combined effect of dbcGMP and FSH showed an inhibition of ODC activity that was equal to that observed with FSH alone.

TABLE 2. Effect of Highly Purified eFSH on ODC Activity in Rat Sertoli Cells*

Treatment	ODC Activity (nmoles/h/mg protein)
DFM-6F	8.5 ± 0.18^{a}
FSH-S-16 (500 ng/ml)	4.2 ± 0.16^{c}
eFSH (5 ng/ml)	$4.6 \pm 0.12^{b,c}$
eFSH (10 ng/ml)	4.7 ± 0.19^{b}

*Rat Sertoli cells in DFM-6F media were treated with FSH-S-16, or eFSH for 4 hours. Data represent mean ± SE (n = 4) and were analyzed by one-way Anova and Duncan's multiple range test. Values with different superscripts within the group differ from each other at $P < 0.001$ to $P < 0.05$.

TABLE 3. Effect of FSH, dbcAMP, MIX, Forskolin, Isoproterenol and dbcGMP on Ornithine Decarboxylase Activity in Sertoli Cells*

Experiment	Treatment	ODC activity (nmoles/h/mg protein)	Percent of Control
I	DFM-6F (control)	5.09 ± 0.08[e]	100
	FSH (0.5 μg/ml)	3.36 ± 0.13[c]	66
	dbcAMP (2.5 mM)	2.75 ± 0.04[a,b]	54
	dbcAMP + FSH	2.81 ± 0.04[a,b]	55
	Butyrate (2.5 mM)	5.45 ± 0.12[e]	107
	Butyrate + FSH	5.58 ± 0.29[e]	110
	MIX (0.1 μg/ml)	4.41 ± 0.16[d]	87
	MIX + FSH	3.22 ± 0.21[b,c]	63
	Forskolin (5 μM)	3.47 ± 0.19[c]	68
	Forskolin + FSH	2.44 ± 0.75[a]	48
II	DFM-6F (control)	5.87 ± 0.26[A]	100
	FSH (0.5 μg/ml)	4.07 ± 0.12[B]	69
	Isoproterenol (5 μM)	3.78 ± 0.40[B]	64
	Isoproterenol + FSH	4.14 ± 0.17[B]	71
	dbcGMP (2.5 mM)	6.00 ± 0.29[A]	102
	dbcGMP + FSH	4.05 ± 0.24[B]	69

*Data represent mean ± SE (n = 4) and were analyzed by one-way Anova and Duncan's multiple range test. Values with different superscripts within the group differ from each other at $P < 0.001$ to $P < 0.05$.

Discussion

Sertoli cells from 18-day-old rats exhibited the highest elevation of cAMP in response to FSH as compared with cells from other age groups (Steinberger et al, 1978). Therefore, Sertoli cells from this age animal were chosen to study the effect of FSH on ODC activity. Addition of FSH to Sertoli cell cultures inhibited ODC activity after 4 hours to 8 hours of treatment. The degree of inhibition was dose-dependent.

The present data differ from observations *in vivo* that intratesticular administration of FSH to 21-day-old rats stimulated testicular ODC activity (Reddy and Villee, 1975). The stimulatory effect of FSH on rat testis ODC activity *in vivo* may result from intercellular interaction or communication between Sertoli cells and other cell types. Studies using co-culture of Sertoli cells and other testicular cell types such as peritubular cells, germ cells or Leydig cells are necessary in investigating this possibility. Although experiments using cells in culture do not always reflect the situation *in vivo*, they may yield valuable information.

Recently, Swift and Dias reported that FSH stimulated (1987), while testosterone (T) inhibited (1986) ODC activity in Sertoli cells derived from 21-day-old rats and cultured in Ham's F-10 media. The present study demonstrated that ODC activity in Sertoli cells from 18-day-old rats is inhibited by FSH (NIH-oFSH-S-15, oFSH-S-16 and highly purified e-FSH). This suppression was observed in three different media, DFM (equal parts of Dulbecco modified Eagle's medium and Ham's F-10 media), DFM-6F and DFM-7F. It was also demonstrated in this laboratory that T stimulated ODC activity in Sertoli cells cultured in DFM-6F or DFM-7F (Tsai et al, 1986). The apparent discrepancies between our results and those of Swift and Dias imply that the Sertoli cell response to FSH may vary with animal age or culture conditions. Further studies are ongoing to probe the opposed observations.

The Sertoli cell is a target cell for FSH action in the testis and responds to FSH with increased accumulation of cAMP (Steinberger et al, 1978; LeGac et al, 1985). The molecular mechanism by which FSH inhibits Sertoli cell ODC activity was studied using dbcAMP, MIX, forskolin and isoproterenol. Like FSH, all of these agents are known to increase intracellular cAMP. Addition of dbcAMP to the cultures suppressed ODC activity while Na-butyrate did not inhibit ODC activity at all. The data suggest that the inhibitory effect was due to cAMP and not butyrate. MIX, a phosphodiesterase inhibitor, increases the level of intracellular cAMP, which in turn results in inhibition of ODC activity. In other systems, however, MIX was shown to increase cellular Ca^{++} levels, which in turn stimulated ODC activity. Although the overall effect of MIX on ODC activity in Sertoli cells was suppressive, it was not as pronounced as the effects of the other agents. Forskolin, a diterpene, increases intracellular cAMP dramatically, presumably by directly linking to the catalytic subunit of adenylate cyclase (Seamon and Daly, 1981). Combined treatment with FSH plus dbcAMP, MIX, forskolin or isoproterenol resulted in inhibition of ODC activity in the Sertoli cells, but the effect was not additive. Our results suggest that FSH inhibition of ODC activity in Sertoli cells is probably mediated by cAMP. On the other hand, addition of dbcGMP to the Sertoli cell culture had no effect on ODC activity and combined treatment with dbcGMP plus FSH showed an inhibition of ODC activity similar to that caused by FSH alone. Therefore, it appears that cGMP is not involved in the FSH inhibition of ODC activity in Sertoli cells.

It is interesting that simultaneous inclusion of Na-butyrate and FSH in the culture abolished the inhibitory effect of FSH on ODC activity (Table 3). This may result from the effect of butyrate on chromatin structure, gene expression or induction of protein

synthesis (Kruh, 1982). Butyrate has been reported to alter chromatin structure and modify chromatin accessibility to certain enzymes (Smith, 1986) and to induce erythroid differentiation (Leder and Leder, 1975) and so forth.

Our observation that fresh media (DFM, DFM-6F or DFM-7F) stimulated Sertoli cell ODC activity agrees with that of Swift and Dias (1986; 1987) using Ham's F-10 media. In general, fresh nutrients such as amino acids stimulate ODC activity in various cell lines, especially after a period of starvation (Abbrachio et al, 1981, Costa et al, 1982). This stimulatory effect precedes the induction of DNA synthesis. However, the primary cultures, unlike established cell lines, usually show some variations from culture to culture. Thus, the absolute Sertoli cell ODC activity and time course of ODC induction may vary within a reasonable range.

In conclusion, FSH exerts a dose- and time-dependent inhibitory effect on ODC activity in Sertoli cells isolated from 18-day-old rats. The mechanism of action is probably mediated by cAMP, but not cGMP. The possibility that the inhibition may result from a stimulatory effect of FSH on some other cellular components affecting the synthesis, degradation or the activity of ODC in the Sertoli cell is not excluded.

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References

Abbrachio MP, Meloni M, Costa M. Cellular effects of ornithine decarboxylase induction in cells maintained with a salts/glucose medium. Life Sci 1981; 28:937–944.

Bousfield GR, Ward DN. Purification of lutropin and follitropin in high yield from horse pituitary glands. J Biol Chem 1984; 259:1911–1921.

Costa M, de Mars M, Lin SN. Ornithine decarboxylase and polyamine levels are reduced in CHO cells deficient in cAMP-dependent protein kinase. Biochem Biophys Res Commun 1982; 107:109–116.

Fozard JR, Part ML, Prakash NJ, Grove J, Schechter P, Sjoerdsma A, Koch-Weser J. L-Ornithine decarboxylase: an essential role in early mammalian embryogenesis. Science 1980; 208: 505–508.

Francis GL, Triche TJ, Brown TJ, Brown HC, Bercu BB. *In vitro* gonadotropin stimulation of bovine Sertoli cell ornithine decarboxylase activity. J Androl 1981; 2:312–320.

Heby O. Role of polyamines in the control of cell proliferation and differentiation. Differentation 1981; 19:1–20.

Janne J, Williams-Ashman HG. On the purification of L-ornithine decarboxylase from rat prostate and effects of thiol compounds on the enzyme. J Biol Chem 1971; 246:1725–1732.

Janne J, Poso H, Raina A. Polyamines in rapid growth and cancer. Biochim Biophys Acta 1978; 473:241–293.

Kobayashi Y, Kupelian J, Maudsley PV. Ornithine decarboxylase stimulation in rat ovary by luteinizing hormone. Science 1971; 172:379–380.

Kruh J. Effects of sodium butyrate, a new pharmacological agent, on cells in culture. Mol Cell Biochem 1982; 42:65–82.

Leder A, Leder P. Butyric acid, a potent inducer of erythroid differentiation in cultured erythroleukemic cells. Cell 1975; 5:319–322.

LeGac F, Attramadal H, Jahnsen T, Hansson V. Studies on the mechanism of follicle-stimulating hormone-induced desensitization of Sertoli cell adenylyl cyclase in vitro. Biol Reprod 1985; 32:916–924.

Levine JH, Nicholson WE, Liddle GW, Orth DN. Stimulation of adrenal ornithine decarboxylase by adrenocorticotropin and growth hormone. Endocrinology 1973; 92:1089–1095.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193:265–275.

MacIndoe JH, Turkington RW. Hormonal regulation of spermidine formation during spermatogenesis in the rat. Endocrinology 1973; 92:595–605.

Madhubala R, Reddy PRK. Stimulation of ornithine decarboxylase activity by prostaglandins in the isolated cells of immature rat testis. Febs Lett 1980; 122:197–198.

Reddy PRK, Villee CA. Stimulation of ornithine decarboxylase activity by gonadotropic hormones and cyclic AMP in testis of immature rats. Biochem Biophys Res Commun 1975; 65:1350–1354.

Russell DH, Byus CV, Manen CA. Proposed model of major sequential biochemical events of a trophic response. Life Sci 1976; 19:1297–1306.

Russell DH. Ornithine decarboxylase: a key regulatory enzyme in normal and neoplastic growth. Drug Metab Rev 1985; 16:1–88.

Seamon KB, Daly JK. Forskolin: a unique diterpene activator of cyclic AMP-generating system. J Cyclic Nucleotide Res 1981; 7:201–224.

Smith P. n-Butyrate alters chromatin accessibility to DNA repair enzymes. Carcinogenesis 1986; 7:423–429.

Steinberger A, Heindel JJ, Lindsey JN, Elkington JSH, Sanborn BM, Steinberger E. Isolation and culture of FSH responsive Sertoli cells. Endocr Res Commun 1975; 2:261–272.

Steinberger A, Hintz M, Heindel JJ. Changes in cyclic AMP responses to FSH in isolated rat Sertoli cells during sexual maturation. Biol Reprod 1978; 19:566–572.

Swift TA, Dias JA. Testosterone suppression of ornithine decarboxylase activity. Endocrinology (suppl.) 1986; (abstr) 243;851.

Swift TA, Dias JA. Stimulation of polyamine biosynthesis by follicle stimulating hormone in serum-free cultures of rat Sertoli cells. Endocrinology 1987; 120:394–400.

Tsai YH, Madhubala R, Shubhada S, Janecki A, Steinberger A, Lin SN. Hormonal effect on the ornithine-decarboxylase activity and polyamine dynamics in rat Sertoli cells. International Conference on Polyamines in Life Science, 1986; 13.