The Mechanism of Sertoli-Germ Cell Interaction[a]

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It has long been hypothesized that Sertoli cell (SC) function is affected by the neighboring germ cells (GC). Using two-dimensional gel electrophoresis followed by autoradiography of the gels (2-D autoradiography), we observe that GC or GC-conditioned medium (GC-medium) causes rapid, dose-dependent, cell-specific changes in ^{32}P incorporation into proteins of cultured SC. Our results indicate that GC interact with SC by way of the phosphatidylinositol (PI) pathway.

For these studies, SC were isolated from young rats and were cultured for three days.[1] SC cultures were completely free of GC. SC were incubated with ^{32}P for 45 min (^{32}P equilibrates with SC ATP in 20 min). After various short-term treatments, SC proteins were prepared and subjected to 2-D autoradiography.[1]

Several proteins showed increased ^{32}P labeling after dibutyryl cyclic AMP (db cAMP) treatment and were also affected by FSH treatment.[1,2] These proteins may be substrates for cAMP-dependent protein kinase (FIG. 1). Treating SC with ionophore A23187 increased ^{32}P labeling of a M_r 26K, pI 5.6 protein (p26), but only if calcium was present in the medium. p26 may be a substrate for calcium/calmodulin-dependent protein kinase (FIG. 2). Treating SC with ionophore plus 12-*O*-tetradecanoylphorbol-13-acetate (TPA) caused increased phosphorylation of a M_r 14K, pI 4.9 protein (p14). p14 may be a substrate for calcium/phospholipid-dependent protein kinase (FIG. 2). Neither p26 nor p14 was affected by db cAMP.

p26 and p14 also showed increased ^{32}P labeling after treatment with GC or GC medium[3] (FIG. 2). After one min exposure to GC, labeling of p26 doubled, and by 5 min labeling increased 5-fold. The response was dose-dependent. p26 seems to be developmentally expressed in SC; it was observed in SC from 18-day-old or older rats, but was not evident in SC from 14-day-old rats. A GC effect on a p26 in the Sertoli cell-derived TR-ST cell line was seen. No GC effect was observed in Chinese hamster ovary (CHO) cells, and both GC and CHO cells lacked p26.

Increased labeling in response to GC or GC medium was also observed in p14. p14 was present in SC from all ages of rats examined and in GC, but a protein with identical 2-D gel mobility was lacking in TR-ST or CHO cells. Current studies in our laboratory include characterization of the GC factor that affects SC, purification of p14 and p26, and elucidation of the PI metabolic pathway in SC.

Our results support two hypotheses: Sertoli cells and germ cells interact by way of the PI pathway, and Sertoli cells have multiple response pathways. Some stimuli may activate one pathway whereas other stimuli may affect distinctly different events.

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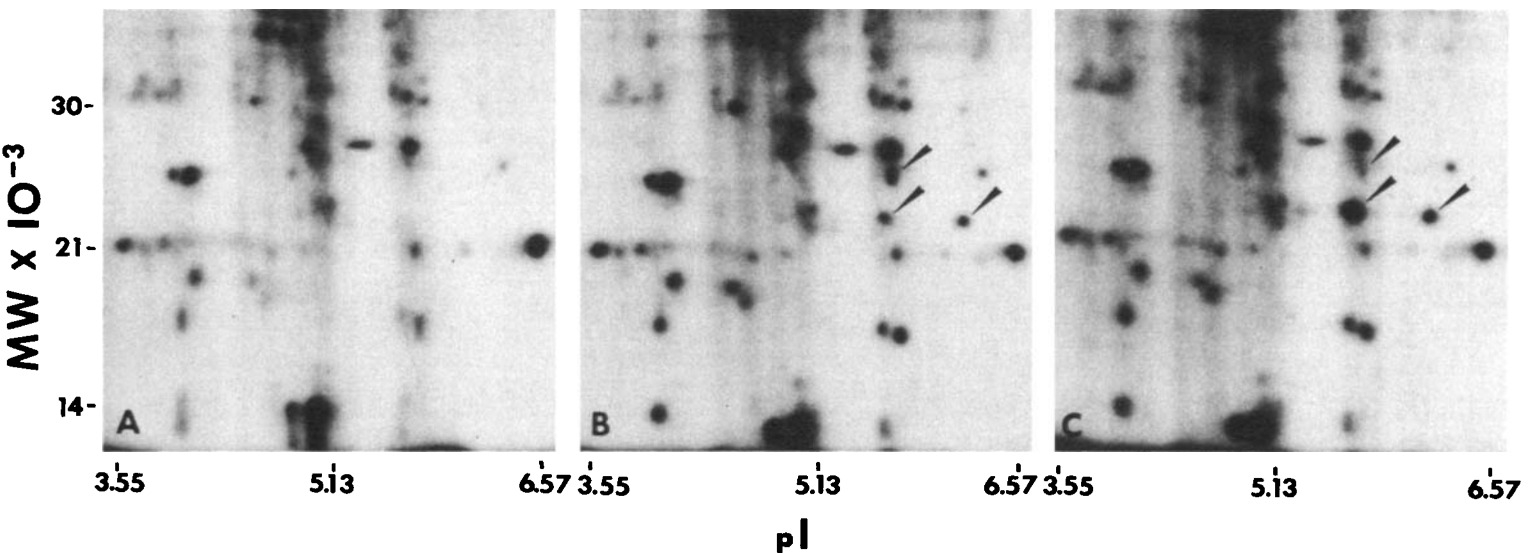

FIGURE 1. Sertoli cells protein phosphorylation in response to dibutyryl cAMP and follicle-stimulating hormone (FSH). SC were cultured, and proteins were prepared for autoradiography as described.[1] In panel **A**, the protein phosphorylation pattern of untreated SC from 20-day-old rats is shown. Treatment of SC with db cAMP (panel **B**) or FSH (panel **C**) results in the phosphorylation of several proteins not phosphorylated in control cultures (arrows). Differences in relative labeling intensity of proteins affected by db cAMP or FSH suggest that FSH does not act by simply increasing cAMP in the cells. Treatments were for 25 min, although treatments for 2–5 min resulted in visible changes in protein phosphorylation. Only a part of the total autoradiogram is shown.

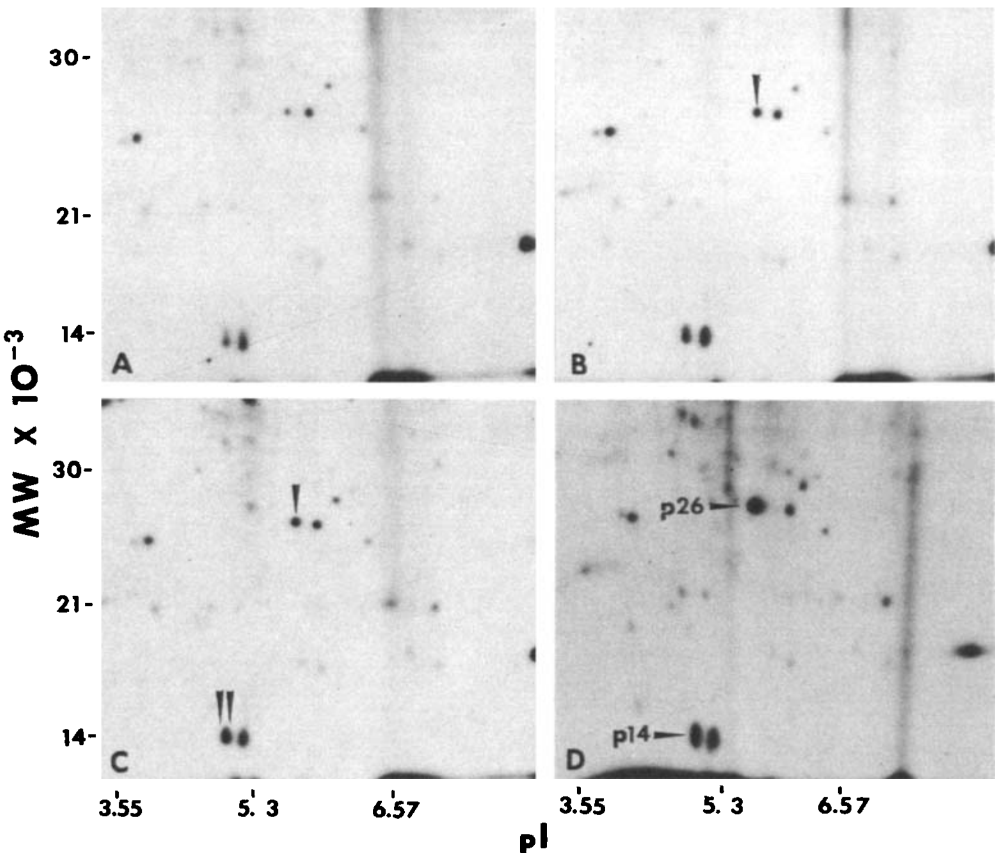

FIGURE 2. Sertoli cell protein phosphorylation in response to A23187, TPA, or germ cells. SC and autoradiograms were prepared as described.[1] Treatments were for 25 minutes. Panel **A** shows the control SC protein phosphorylation pattern. When SC were exposed to ionophore A23187 with calcium in the medium, p26 showed increased ^{32}P labeling (arrow, panel **B**). When SC were exposed to A23187 and TPA together, an additional phosphoprotein, p14, showed increased incorporation of ^{32}P (double arrows, panel **C**). Treatment of SC with GC or GC medium also resulted in increased phosphorylation of p26 and p14 (panel **D**). Equal increases in ^{32}P incorporation were measured after 5 min GC treatment, and statistically significant increases in ^{32}P incorporation into p26 and p14 were measured after only 60 seconds exposure to GC. Only a part of each autoradiogram is shown. Differences in overall labeling intensity between autoradiograms shown in FIGURES 1 and 2 result from the fact that the films were exposed to the 2-D gels for differing lengths of time.

REFERENCES

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