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EVIDENCE THAT POTASSIUM CHANNELS REGULATE PROLACTIN SECRETION IN GH_4C_1 CELLS BY CAUSING EXTRACELLULAR CALCIUM INFLUX

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Summary Tetraethylammonium (TEA), a K^+ channel blocker, induced prolactin (PRL) secretion in GH_4C_1 cells in a dose-dependent manner when applied at a concentration from 1-20 mM. During continuous exposure to TEA, a significant increase in PRL secretion occurred by 20 min and the response was sustained until the end of a 60-min exposure. Blocking Ca^{2+} influx by employing a Ca^{2+}-depleted medium or the Ca^{2+} channel blocker, nifedipine, prevented induction of PRL secretion by 20 mM TEA. Preincubation of the cells for 10 min with 20 mM TEA did not inhibit PRL secretion induced by thyrotropin-releasing hormone (TRH), phorbol 12-myristate 13-acetate (TPA) or by cell swelling produced by 30% medium hyposmolarity, but significantly depressed that induced by depolarizing 30 mM K^+. $BaCl_2$, another K^+ channel blocker, had the same effect on PRL secretion as TEA. The data suggest that blocking K^+ channels may cause membrane depolarization, thereby inducing Ca^{2+} influx which is a potent stimulus for PRL secretion in GH_4C_1 cells.

Introduction Evidence is increasing that cell swelling and cell volume regulation are important modulators of hormone secretion (1-3). Thyrotropin-releasing hormone (TRH) induces a membrane potential change in rat pituitary tumor-derived GH cells, causing a brief hyperpolarization followed by an action potential (4). The membrane potential change is an important factor in regulating hormone secretion (5). Depolarizing 30 mM medium K^+ also causes membrane depolarization which is followed by hormone secretion (6). K^+ channels play an important role in both the regulatory volume decrease following osmotically induced cell swelling (7) and in repolarization of the cell membrane after depolarization (8) and thus may have a critical function in the physiological regulation of hormone secretion. In the present study, we used tetraethylammonium (TEA), which binds with millimolar affinity to the intracellular opening of the K^+ channel pore, to probe the role of K^+ channels in the regulation of prolactin (PRL) secretion. GH_4C_1 cells were chosen because they are a homogeneous, well-studied clonal pituitary tumor-derived cell line whose plasma membrane can be depolarized by 30 mM K^+. These cells have voltage-dependent Ca^{2+} channels (VDCC) (9) and cell swelling-induced secretion has been extensively studied in them (3,10).

Materials and Methods

Cell Preparation: GH_4C_1 cells were grown in monolayer in Ham's F-10 medium (GIBCO) supplemented with 15% horse serum and 2.5% fetal calf serum (GIBCO) at 37 C ($F\text{-}10^+$). Four

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days prior to the experiment the cells were harvested with versine and cultured in 24-well multiwell tissue culture plates, each well containing 10^5 cells. For the experimental studies, the cells in each well were washed once and incubated with Krebs-Ringer-bicarbonate-HEPES buffer (KRBH) for one hour before the experiment. Cells were incubated for 5-60 min with medium containing various test substances. The media samples were stored at -20 C until assayed for PRL. At the end of the experiment, >95% of the cells were viable as measured by their ability to exclude 0.02% trypan blue. To evaluate the possibility that the PRL increase in the media may have been caused by cell detachment, we compared centrifuged and uncentrifuged media. There was no significant difference between the two groups ($p>0.05$).

Preparation of Drugs and Medium: The drug solutions were prepared immediately before use. Tetraethylammonium (TEA), nifedipine, phorbol 12-myristate 13-acetate (TPA), and thyrotropin-releasing hormone (TRH) (Sigma), and $BaCl_2$ (J.T. Baker) were dissolved in KRBH (NaCl 140 mM; KCl 5 mM; $MgSO_4$ 1.2 mM; KH_2PO_4 1.2 mM; glucose 6 mM; HEPES (N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]) 10 mM; $CaCl_2$ 1.5 mM; and 0.1% BSA) or "Ca^{2+}-free" KRBH with the same composition as KRBH except the NaCl concentration was 142.2 mM, $CaCl_2$ was omitted, and 0.1 mM EGTA was added.

Prolactin Assay: Medium PRL was measured by double-antibody radioimmunoassay using protocols and specific reagents supplied by the National Hormone and Pituitary Program of the NIDDK. The samples from each experiment were analyzed in a single assay to avoid interassay variation. The intra-assay coefficient of variation for all assays was <8%. The upper and lower range of sensitivity in all assays was between 1 and 50 ng/ml for PRL.

Statistics: The results were analyzed with Student's *t*-test, the Newman-Keuls multiple comparison test, or linear regression and correlation, as indicated. $P<0.05$ was considered statistically significant. All values are expressed as Mean ± SE.

Results

Effect of TEA on PRL secretion: Incubation of GH_4C_1 cells with 1-20 mM TEA for 30 min induced PRL secretion in a dose-dependent manner ($r=0.965$; $p<0.01$). The minimum effective dose of TEA was 5 mM and the EC_{50} was 10 mM (Fig 1). $BaCl_2$, another K^+ channel blocker, at a concentration of 1 mM had an effect similar to TEA (data not shown).

Temporal dynamics of TEA-induced PRL secretion: During continuous exposure of GH_4C_1 cells to 20 mM TEA, a significant increase in PRL secretion was induced by 20 min and the increased response was sustained for the rest of the 60 min incubation ($p<0.05$). (Fig 2).

Effect of extracellular Ca^{2+} concentration and the Ca^{2+} channel blocker, nifedipine, on TEA-induced PRL secretion: When Ca^{2+} influx was blocked by depleting the medium Ca^{2+} or employing 2 μM nifedipine, basal PRL secretion was significantly decreased and TEA-induced PRL secretion was completely abolished (Fig 3).

Effect of TEA on PRL secretion induced by hyposmolarity, TRH, TPA or depolarizing K^+: All of these stimuli induced a significant increase in PRL secretion. Ten min preincubation with 20 mM TEA before and during exposure to these stimuli decreased secretion induced by depolarizing K^+ by 50% but secretion induced by TRH, TPA or hyposmolarity was not affected (Fig 4).

Discussion Our data suggest that K^+ channels play a role in the regulation of PRL secretion in the pituitary-tumor derived GH_4C_1 cell line. Although TEA and $BaCl_2$ have different structures,

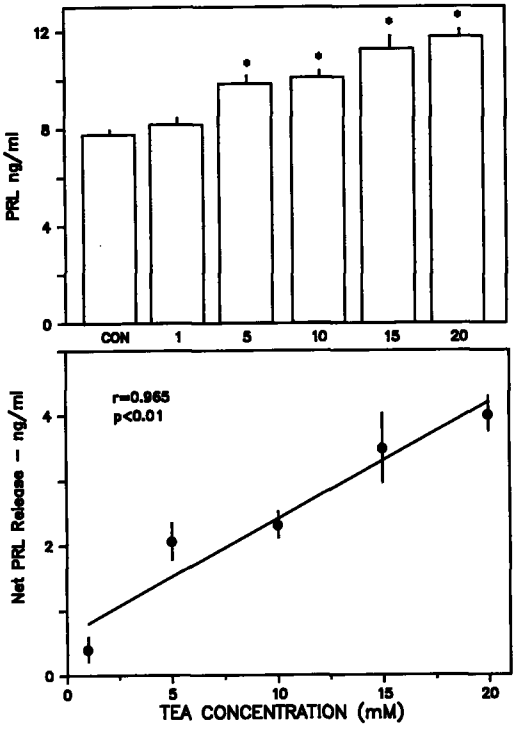

Fig.1. Effect of graded concentrations of TEA on PRL secretion. Each bar shows the Mean and SE of PRL secretion by 4 wells over a 30 minute period. CON=control; the mM concentration of TEA is given below each of the other bars. The lower panel shows the net (TEA - mean of control) from the same experiment shown in the upper panel. *=p<0.05 vs. control (Newman-Keuls).

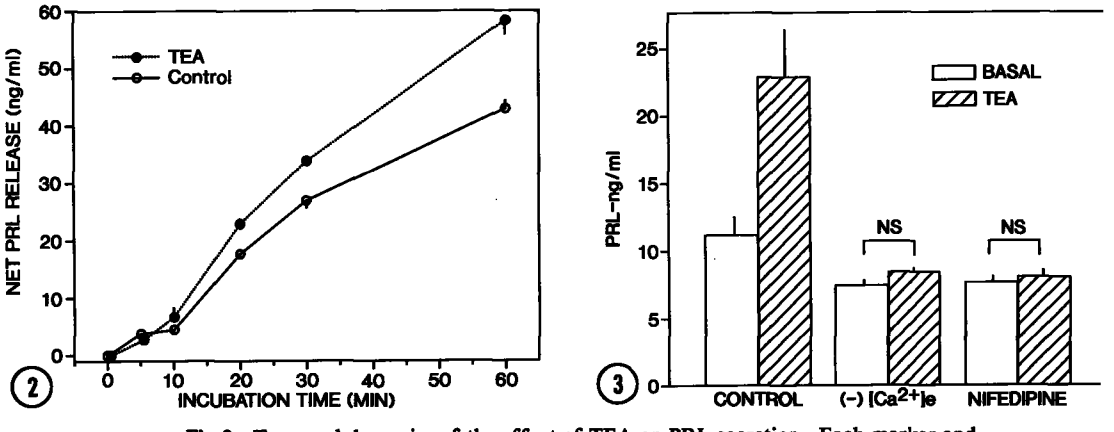

Fig.2. Temporal dynamics of the effect of TEA on PRL secretion. Each marker and vertical line show the Mean and SE of PRL release in 4 wells. Where no vertical line is shown, SE was smaller than the marker. TEA = 20 mM TEA added to the medium at 0 time. There was no difference between the TEA and control groups ($p>0.05$) at 0-10 min incubation, but there were significant differences between TEA and control at 20, 30, and 60 min ($p<0.05$, Newman-Keuls).

Fig.3. Effect of reducing Ca^{2+} influx with Ca^{2+}-depleted medium or with nifedipine on PRL secretion induced by 20 mM TEA. Cells were incubated in control medium (CONTROL), Ca^{2+}-depleted medium ((-)$[Ca^{2+}]_e$), or medium containing 2 μM nifedipine (NIFEDIPINE) for 30 min. Basal secretion is indicated by open bars and induced secretion by hatched bars. Mean and SE of 8 wells are indicated by each bar and vertical line. There was a significant stimulation of PRL secretion by TEA in the control medium ($p<0.05$, Newman-Keuls) but no significant stimulation by TEA in the groups in which Ca^{2+} influx was blocked. (NS = $p>0.05$).

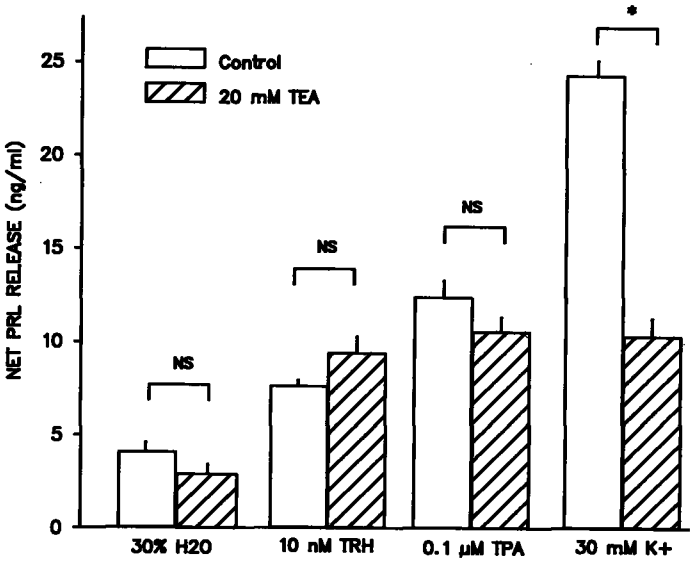

Fig.4. Effect of TEA on PRL secretion induced by hyposmolarity, TRH, TPA and depolarizing K^+. Cells were incubated in wells with normal medium alone or medium with 20 mM TEA for 30 min. Each bar and vertical line indicates the Mean and SE of 4 wells. Net PRL release was calculated as induced release (with or without TEA) - control (with or without TEA). The open bars indicate the effect of the various stimuli alone and the hatched bars indicate the effect of the stimuli in cells incubated in medium containing 20 mM TEA, including 10 min preincubation. *=$p<0.05$, Student's t; NS=$p>0.05$.

both block K^+ channels and induce PRL secretion. This suggests that neither has some peculiarity in structure which inhibits PRL secretion but that they both act through the common pathway of blocking K^+ channels.

As far as we are aware, this is the first report of a role of K^+ channels in pituitary secretion. The effective concentration range of the K^+ channel blockers in our experiments is compatible with the data of other investigators (11). The K^+ channel-blockers inhibited PRL secretion induced by depolarizing K^+ but did not inhibit PRL secretion induced by TRH, TPA or hyposmolarity. This indicates that the effects of the K^+ channel-blockers are not due to nonspecific toxicity to the cells. This is further confirmed by the observation that >95% of the cells were undamaged as shown by trypan blue exclusion at the end of the experiments.

The mechanism by which K^+ channel blockers affect PRL secretion is not delineated. These agents promote catecholamine (CA) release from chromaffin cells and from sympathetic nerve terminals (12,13). Changes in membrane potential and $[Ca^{2+}]_i$ concentration have been suggested as the mechanisms by which TEA induces CA secretion (13). The endocrine cells of the adenohypophysis are electrically excitable (4). A change in membrane potential may play an important role in regulating Ca^{2+} influx (5) and exocytosis (14). K^+ channels are involved in the modulation of membrane potential; in lactotrophs dopamine inhibits PRL secretion by increasing voltage-dependent K^+ currents (15). In GH_3 cells, somatostatin may inhibit secretion by activating a subset of K^+ channels (16,17).

Because of the above considerations we postulated that directly altering K^+ channel activity might influence adenohypophyseal secretion. It is widely accepted that a rise in cytosol

Ca^{2+} can initiate PRL secretion (18). In our studies, blocking Ca^{2+} influx by depleting medium Ca^{2+} or with nifedipine prevented TEA activation of PRL secretion. Depleting medium Ca^{2+} also depresses TEA induction of catecholamine secretion (13).

Nifedipine inhibits the influx of Ca^{2+} through L-type Ca^{2+} channels (Ca^{2+} channels that mediate long-lasting Ca^{2+} currents) in GH_4C_1 cells (19). Since nifedipine blocked TEA-induced PRL secretion in our studies, it seems highly probable that L-type Ca^{2+} channels are involved. In somatotrophs, spontaneous oscillations of depolarization of the plasma membrane occur (20). K^+ channels participate in restoring cell membrane potential through repolarization after excitation (8,21); K^+ channel blockers are postulated to inhibit or delay the repolarization. From these reports, our data indicate that K^+ channel-blockers may cause membrane depolarization in GH_4C_1 cells, resulting in Ca^{2+} influx through L-type Ca^{2+} channels and thus stimulating PRL secretion.

A variety of different secretagogues incubated with GH_4C_1 cells in static culture for 1 h stimulate >50% of the total PRL secretion within the first 10 min. (*e.g.* TRH (22,23), depolarizing K^+ (18) and hyposmolarity (1,24)). This corresponds temporally to the initial high-amplitude secretagogue-stimulated secretory burst seen with perifused GH_4C_1 and other pituitary cell types (23). In our experiments TEA did not have a significant effect on PRL secretion until after the first 10 min. However, there was progressive stimulation at 20 to 60 min. This indicates that TEA has a slow onset and sustained effect in stimulating PRL secretion, making it more resemble vasoactive intestinal peptide (25) than TRH, K^+, or cell swelling. TEA had no effect on TRH-induced PRL secretion, also suggesting that TEA and TRH induce PRL secretion by different mechanisms. The K^+ current across the plasmalemma, which is blocked by both TEA and $BaCl_2$ (11), may play a role in regulating PRL secretion during long periods of stimulated secretion.

Since K^+ efflux participates in the regulatory cell volume decrease following osmotic cell swelling (7), it is possible there may be some interrelation between K^+ efflux induced by cell swelling and secretion. However, TEA cannot block secretion induced by osmotic cell swelling. Our results indicate that K^+ channels do not participate in cell swelling-induced PRL secretion in GH_4C_1 cells. It seems clear that TEA has a different relationship to K^+-induced secretion than to secretion induced by cell swelling since TEA inhibited secretion stimulated by depolarizing K^+. Whatever the mechanism of their effect on secretion eventually turns out to be, K^+ channel-blockers may serve a useful role as a new tool for studying mechanisms of exocytotic secretion.

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