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Off-target effects of MEK inhibitors

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Abstract

The mitogen-activated protein kinases (MAPKs) ERK1/2 regulate numerous cellular processes including gene transcription, proliferation, and differentiation. The only known substrates of the MAP2Ks MEK1/2 are ERK1/2; thus, the MEK inhibitors PD98059, U0126, and PD0325901 have been important tools in determining the functions of ERK1/2. By using these inhibitors and genetically manipulating MEK, we find that ERK1/2 activation is neither sufficient nor necessary for regulated insulin secretion from pancreatic beta cells or epinephrine secretion from chromaffin cells. We show that both PD98059 and U0126 reduce agonist-induced calcium entry into cells independently of their ability to inhibit ERK1/2. Caution should be used when interpreting results from experiments using these compounds.

ERK1/2 have many cellular roles including regulating glucose-induced insulin gene transcription in pancreatic beta cells [1]. It is clear that many insulin secretagogues induce ERK1/2 activation [1]. As beta cells secrete insulin in response to secretagogues, biosynthetic processes including insulin gene transcription, which is dependent on ERK1/2 activation, are engaged to replenish secreted hormone. Studies investigating the role of ERK1/2 in insulin secretion have been performed with conflicting conclusions [2-6]. Many investigators have used the MEK1/2 inhibitors PD98059, U0126, and PD0325901 to investigate ERK1/2 functions [7, 8]. U0126 suppressed the expression of an AP-1 driven luciferase reporter in COS-7 cells maximally at a dose of between 10 and 20 μM and 40 μM PD98059 inhibited c-Fos phosphorylation[8, 9] PD0325901 can inhibit the phosphorylation of downstream targets of ERK1/2 at 10 nM [10].

We observed that blockade of the ERK1/2 pathway with U0126, an inhibitor of the upstream kinases (MEK1/2) reduced amino acid-induced ERK1/2 activation and insulin secretion, suggesting that there is a component of secretion that is dependent upon ERK1/2. However, the other MEK1/2 inhibitors PD98059 and PD0325901 did not inhibit amino acid-induced insulin secretion, despite reducing ERK1/2 activation (Figures 1A, B). Because the role of ERK1/2 in insulin secretion has been in question in the literature [2-5], we evaluated this possibility more thoroughly. To determine if prolonged activation of ERK1/2 was sufficient, we tested effects of constitutively active MEK1 on insulin secretion and found no change in secretion in spite of elevated ERK1/2 activity (Figures 1C, D). We did not observe a change

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Supporting Information: Detailed Methods and additional data This material is available free of charge via the Internet at http://pubs.acs.org.

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in basal insulin secretion with constitutively active MEK1 (Figure 1 of Supporting Information).

On further analysis of an ERK1/2 requirement for secretion, we found that two commonly used MEK1/2 inhibitors interfered with calcium homeostasis in β cells (Figure 2A). The increase in intracellular free calcium induced by amino acids was strongly inhibited by PD98059 and partially prevented by U0126 (Figure 2A). A MEK1/2 inhibitor more recently available, PD0325901, even at a concentration of 500 nM had no effect on calcium changes induced by amino acids (Figure 2A). Examining the average basal free calcium prior to addition of amino acids revealed that PD98059 strongly decreased this value while U0126 slightly reduced it (Figure 2B).

We used bovine adrenal chromaffin cells stimulated with the secretagogue nicotine or depolarized with KCl to further characterize effects of PD98059. In these cells ERK1/2 activation paralleled norepinephrine secretion very well over several minutes (Fig. 2A of the Supporting Information). PD98059 blocked activation of ERK1/2 at 2 μM, but did not affect secretion induced by nicotine or depolarization until concentrations above 10 μM (Figure 2B of the Supporting Information and Figure 3A). Since secretion in chromaffin cells is absolutely dependent on the entry of extracellular Ca^{2+}, we examined if this process was impaired by concentrations of PD98059 which inhibit secretion. ^{45}Ca uptake was measured in cells treated with nicotine or 59 mM KCl [11]. ^{45}Ca influx induced by nicotine or KCl was not reduced at concentrations of PD98059 lower than 15 μM; these concentrations are sufficient to prevent ERK1/2 activation, but have no effect on secretion (Figure 3B). However, at higher PD98059 concentrations, ^{45}Ca uptake was inhibited in a dose-dependent manner, reaching 80% and 50% inhibition at 75 μM PD98059 for nicotine and KCl-stimulation, respectively (Figure 3B). These findings are consistent with those in β cells and suggest the generality of off-target actions of PD98059.

We reinvestigated the potential role of ERK1/2 in secretory responses and found that activation of the kinases is not sufficient for secretion from pancreatic β cells. In fact, although kinase activation parallels secretion, it is not necessary for secretion from pancreatic β cells or from adrenal chromaffin cells. Previous researchers using 100 μM PD98059 concluded that ERK1/2 activation was necessary for insulin secretion induced during the first 5 min after MIN6 cells were stimulated with glucose[6]. However, the inhibitory effect on insulin secretion was potentially due to a decrease in calcium entry, which occurred when we used a dose of 20 μM PD98059. Reinvestigation of ERK1/2 effects on secretion led us to examine effects of three MEK inhibitors and discover off-target artifacts of two commonly used compounds on calcium homeostasis. Although these compounds have been of considerable value over nearly twenty years, some off target and toxic effects of U0126 and PD98059 have been reported, generally limiting their use to short term studies [4, 12, 13]. These compounds affect AMPK activity and U0126 has recently been shown to interfere with mitochondrial respiration [12-14].

The concentrations of these inhibitors that activate AMPK are similar to the ones that we observe inhibit calcium entry. Thus, it possible that these two off-target effects are mechanistically connected. On the other hand, PD0325901 did not perturb calcium homeostasis and did not interfere with subsequent activation of ERK1/2 even after two days of exposure (Zaganjor, E. et al., in revision). Given our data reported here and the recent report in which doses of both PD98059 and U0126 commonly used to inhibit MEK1/2 reduced mitochondrial respiration [14], results from experiments using these drugs should be interpreted cautiously.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

aa	amino acids
KRBH	Krebs-Ringer Bicarbonate Solution

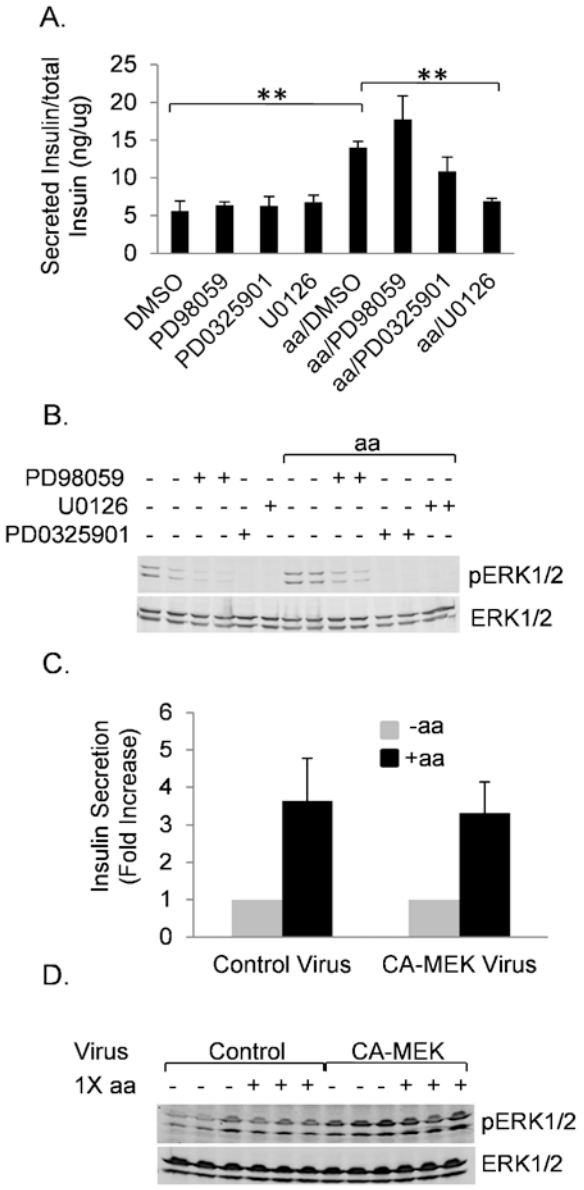

Figure 1.

ERK1/2 activity is not sufficient or necessary for amino acid-induced insulin secretion. (A) MIN6 cells were incubated in KRBH for 2 h and 45 min before being pretreated with DMSO, 20 μM PD98059, 500 nM PD0325901, or 10 μM U0126 for 15 min. Cells then were stimulated with 1X aa for 30 min before the KRBH was collected and the cells were lysed. Insulin content was measured in both the lysates (total insulin) and KRBH (secreted insulin) with an ELISA (Materials and Methods). Data are mean values +/- sem (bars) representative of three independent experiments each done in triplicate. **$p < 0.01$, two-tailed Student's t test. (B) SDS-PAGE and immunoblotting on the lysates from (A). (C) MIN6 cells were infected with either a beta-gal control adenovirus or a virus encoding constitutively active MEK (CA-MEK). 24 h later, cells were treated incubated in KRBH for 2 h and 30 min before being stimulated with 1X aa. 30 min later, KRBH was collected cells were lysed and insulin content was measured as in (A). The data are presented as the fold increase in insulin secretion induced by 1X aa. Data are mean values +/- standard deviation

(bars) from two experiments each carried out in triplicate. (D) Immunoblots from the cell lysates in (C).

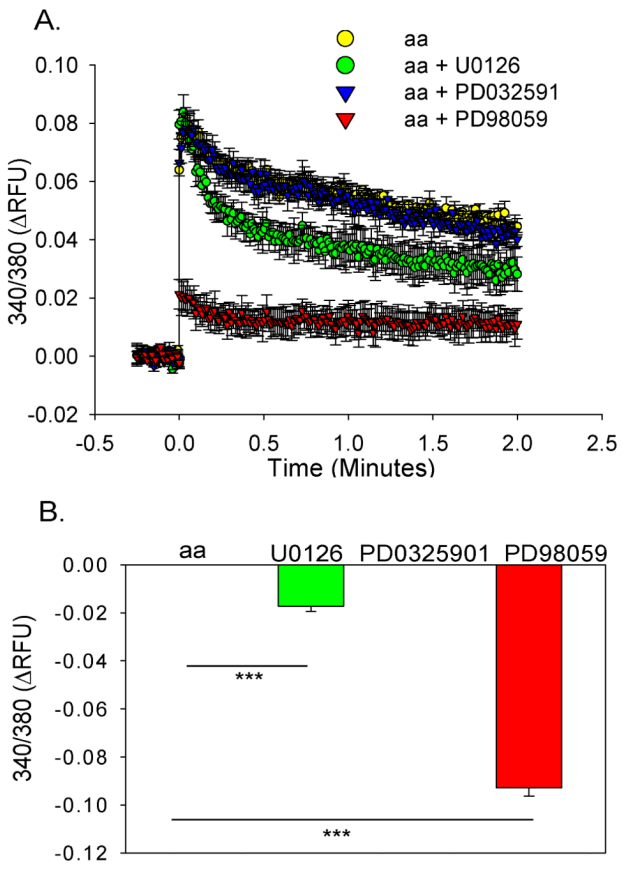

Figure 2.

Uo126 and PD98059 inhibit calcium entry independently of ERK1/2 inhibition. (A-B) MIN6 cells were placed in KRBH without aa, loaded with fura-2, and pretreated with the indicated concentrations of the indicated inhibitors or DMSO (vehicle) for 30 min prior to being stimulated with aa. (A) Baseline ratios from each condition before aa addition were averaged and then subtracted from each of the points in the respective condition to correct for the significant differences in basal free calcium levels. (B) Baseline free calcium levels prior to aa stimulation. (A-B) Data are the mean +/- sem of the 340/380 values from three experiments, each done in triplicate (Materials and Methods). ***$p < 0.001$, two-tailed Student's t test.

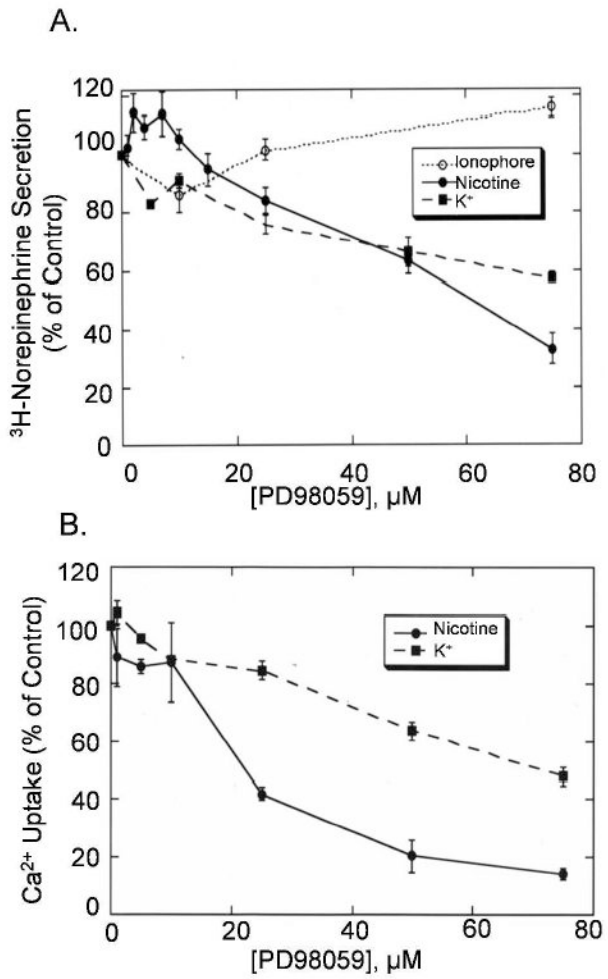

Figure 3.

PD98059 blocks norepinephrine release from chromaffin cells independently of ERK1/2 inhibition. (A) Cells were stimulated with either 10 μM nicotine, 59 mM KCL, or 10 μM ionomycin for 10 minutes in the presence of concentrations of PD98059 (up to 75 μM). 3H-norepinephrine release is expressed as a percent of the release in the absence of the drug. Data are mean +/- sem (bars) values from five (nicotine), four (K+), and three (ionomycin) experiments, each carried out in triplicate. (B) 45Ca2+ uptake was measured after 10-minute stimulations with either 10 μM nicotine or 59 mM KCl as a function of PD98059 concentration. The data are presented as a percent of the uptake in the absence of the drug. The uptake in the absence of PD98059 varied between 1.5-2 fmol 45Ca/cell. Data are +/- sem values from three experiments for each secretagogue, each carried out in triplicate.