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Absence of effect of culture duration on glucose-activated alterations in intracellular calcium concentration in mouse pancreatic islets

We would like to comment on the recent report by Gilon et al. [1] regarding their findings with cultured mouse islets.

The rise in intracellular calcium ($[Ca^{2+}]_i$) induced by glucose in islet of Langerhans beta cells, and its' linkage to insulin secretion is firmly established [2]. Based on our own and other studies, a highly reproducible triphasic pattern of changes in $[Ca^{2+}]_i$ occurs in mouse islets in response to raising the extracellular glucose concentration. These comprise an initial reduction in $[Ca^{2+}]_i$, as in "These conprize on initial reduction in $[Ca^{2+}]_i$ [3, 4], which we have termed phase 0, due to the activation of endoplasmic reticulum Ca^{2+}-ATPase-dependent sequestration of Ca^{2+} [5], a transient rise in $[Ca^{2+}]_i$ (phase 1) caused by a combination of Ca^{2+} store mobilization [3, 6] and Ca^{2+} store-depletion activated Ca^{2+} influx [7, 8]) and regular oscillations in $[Ca^{2+}]_i$ (phase 2), that vary in frequency from 0.5–3 min^{-1}, and primarily represent Ca^{2+} influx [3, 7–11]. Recently, Gilon et al., have reported that culturing mouse islets for as little as 3 days causes a gross alteration in this pattern of Ca^{2+} changes; the phase 0 decreases in magnitude and duration and the distinct phase 1 transient and phase 2 oscillations are replaced by a sustained Ca^{2+} rise [1]. No explanation for these changes in responsiveness was offered.

Our studies have failed to reproduce any aspect of these results: in our original description of the triphasic alterations in $[Ca^{2+}]_i$ to glucose [3], we studied islets cultured from periods ranging from 6–12 days and obtained results qualitatively equivalent to the acute/short-term culture (i.e. less than

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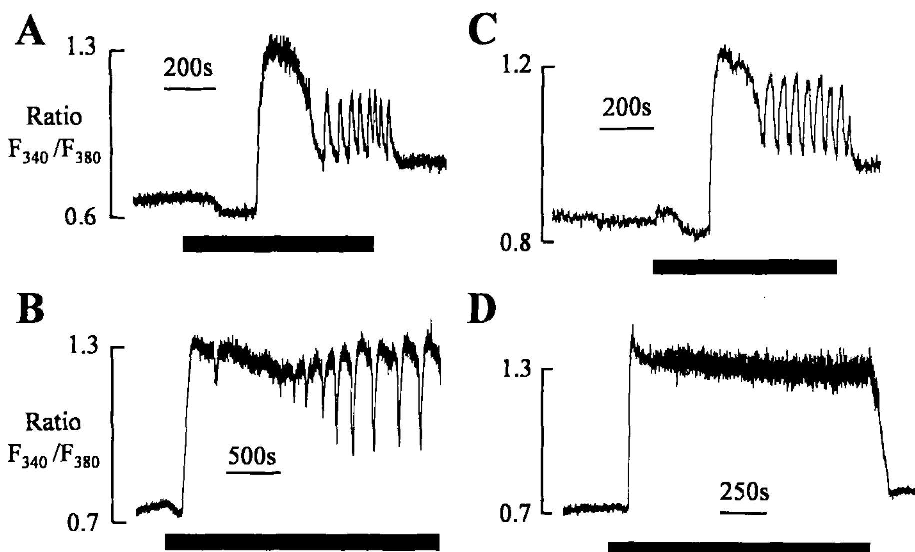

Fig. 1. (A–D) Lack of influence of culture duration on the changes in $[Ca^{2+}]_i$ occurring in mouse islets stimulated by an increase in glucose. The islets were cultured in RPMI 1640 medium containing 11.6 mmol/l glucose, 100 μU/ml penicillin and 100 μg/ml streptomycin for up to 14 days. They were then loaded with fura-2, transferred to the recording chamber, and perifused with a medium containing 2 mmol/l glucose for about 10 min before challenging with 12 mmol/l glucose (filled bar). More complete experimental details appear elsewhere [3, 5, 7, 8, 12, 14]. Shown in the upper panels are the responses following long-term (10 day, **A**) and short-term (2 day, **C**) culture of islets isolated from C57BL/6J and C57BL/KsJ mice, respectively. The lower panels show the responses following long-term (7 day, **B**) and short-term (1 day, **D**) culture of islets isolated from C57BL/6J-ob/ob (ob/ob) and C57BL/KsJ-db/db (db/db), respectively. Note that the only responses that resemble those reported by Gilon et al. [1] are those from the obese hyperglycaemic ob/ob and diabetic db/db islets, and that these responses are independent of culture duration. Control animal islet $[Ca^{2+}]_i$ responses to 12 mmol/l glucose are unaffected by long-term culture

3 days) results of Gilon et al. [1]. In subsequent studies, the length of culture period ranged from 3–12 days [7, 8, 12], and no change in glucose responsiveness over this time period of the type reported by Gilon et al. was detected. Our studies were carried out using islets isolated from the C57BL/KsJ strain of mouse, whereas those of Gilon et al. utilized NMRI mice, raising the possibility that strain-specific differences were responsible for the divergent results. As shown in Figure 1, we extended these observations to another strain of mouse, the C57BL/6J. Even in this mouse strain, we were unable to observe the alterations in long-term culture reported by Gilon et al. [1]. The relevance of their findings even with respect to the NMRI mice is brought into question by previous studies demonstrating no effect of 7-day culture in 10 mmol/l glucose on insulin secretory responsiveness of NMRI islets [13]. In fact, the only situation in which we were able to observe the reported types of aberrant $[Ca^{2+}]_i$ responses to glucose was in islets isolated from the diabetic db/db mouse [14] and to a lesser extent in islets from the obese hyperglycaemic ob/ob mouse (Fig. 1 B and D). As in the case of the control islets, the abnormal responsiveness to glucose was observed immediately (1–2 days), and remained consistent throughout the period of study (10 days).

The isolation and culture conditions employed by Gilon et al. [1] were essentially identical to our own. The only experimental difference was the concentration of stimulatory glucose employed; 15 mmol/l glucose vs the 11–12 mmol/l glucose utilized by most groups. Our studies have shown that at 20 mmol/l glucose (again irrespective of culture duration) a sustained rise in Ca^{2+} is produced [14], due to the non-oscillatory depolarization induced by this supramaximal concentration of glucose (normal glucose levels under fed conditions in mice and man rarely exceed 12 mmol/l). Therefore, it is conceivable that a subtle increase in glucose sensitivity over the culture period might partially explain the loss of Ca^{2+} oscillations reported by Gilon et al. [1]. It would not, however, explain the disappearance of the phase 0 response, which in db/db mice is associated with a pathological decrease in endoplasmic reticulum Ca^{2+} ATPase levels [4]. Thus, the similarity of the changes in $[Ca^{2+}]_i$ in response to glucose reported by Gilon et al. [1] in the NMRI islets to those we have reported in ob/ob and db/db islets would suggest that the NMRI islets are metabolically compromised.

Yours sincerely,
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Response from the authors

Dear Sir,

Drs. Roe et al. disagree with our recent report [1] that glucose-induced oscillations of $[Ca^{2+}]_i$ in beta cells are altered when the islets (from NMRI mice) are cultured for 3–4 days. In their experience, the pattern of $[Ca^{2+}]_i$ changes induced by glucose is highly reproducible in islets (from C57BL/KsJ and C57BL/6J mice) cultured for 3–12 days, and similar to what we have observed with overnight cultured NMRI mouse islets [1–3]. Because the response of NMRI mouse islets cultured for 4 days resembles that of ob/ob and db/db mouse islets cultured for only 1–2 days [4], Roe et al. suggest that NMRI mouse islets are metabolically compromised and that our findings are pertinent only to the islets of the particular strain that we use.

We have tested this hypothesis in experiments using one strain studied by Roe et al., C57BL/6J mice. The islets were

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