

## REPLY

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# Reply to Comments on the Article “[6]-Shogaol Induced Calcium Signal in Rat Insulinoma Cells”

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Dear Sir,

The  $[Ca^{2+}]_i$  increase obtained by 1  $\mu$ M [6]-shogaol was on the average greater than that obtained by 10 mM glucose. However, this observation *per se* is not important because  $[Ca^{2+}]_i$  increases by both [6]-shogaol and glucose were dose dependent. Thus, comparing the effect of one particular concentration of [6]-shogaol with the effect of one particular concentration of glucose is not so useful. It should be noted that we used rat insulinoma cells which are less glucose responsive compared to the primary  $\beta$ -cells.

$[Ca^{2+}]_i$  mean ( $\pm$ SEM) increase by the maximally effective concentration of [6]-shogaol was  $493 \pm 299$  (n=4). There was no need to perform statistical test of significance because there was no increase in  $[Ca^{2+}]_i$  in the control experiments where [6]-shogaol was not used. There was also no need to compare pre-drug and post-drug  $[Ca^{2+}]_i$  statistically, because in all the experiments, the  $[Ca^{2+}]_i$  returned to the baseline after washout of the drug. One should not

misuse statistics just because it can be performed easily.

The graphical representations on the y axis do not follow a fixed interval. This is because of the fact that we measured the ratio F340/F380 which is not linearly related to the  $[Ca^{2+}]_i$ .  $[Ca^{2+}]_i$  was calculated from the F340/F380 ratio (which is linear) using the calibration data, and the Grynkiewicz formula [1]. These are mentioned in the methods section.

It is well known that carbachol increases  $[Ca^{2+}]_i$  mainly by activating the phospholipase C, formation of inositol 1,4,5-trisphosphate, and release of  $Ca^{2+}$  from the endoplasmic reticulum. KCl increases  $[Ca^{2+}]_i$  by depolarization of the plasma membrane, and activation of the voltage-gated  $Ca^{2+}$  channels. These basic facts are text book knowledge and they hardly require any reference [2].

At this stage, we do not think that our observations reported in this paper are of major importance in the context of understanding the pathogenesis or treatment of human diabetes.

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**Key words** Calcium Signaling; Insulin-Secreting Cells; Islets of Langerhans; shogaol

**Abbreviations**  $[Ca^{2+}]_i$ : cytoplasmic free  $Ca^{2+}$  concentration

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**Conflict of interest** The authors have no potential conflict of interest

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