REPLY TO BRENKER ET AL.:

The plant triterpenoid pristimerin inhibits calcium influx into human spermatozoa via CatSper

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In the letter by Brenker et al. (1), the authors assessed actions of various steroids toward the human calcium channel of sperm (CatSper). The experiments, carried out with calcium imaging, are minimally supplemented by electrophysiology. The authors show that all tested steroids activate CatSper with different efficiencies. However, the plant triterpenoids pristimerin and lupeol fail to interfere with calcium influx. These data contradict our previously published results (2).

CatSper is the principal calcium channel of spermatozoa that mediates calcium influx into the sperm tail. When studying CatSper with electrophysiology, inward currents (influx of positive ions into the cell) are of particular interest. However, outward currents (efflux of positive ions) are elicited only under nonphysiological potentials and are not necessarily CatSper-mediated. To study CatSper inward currents, a driving force must be generated experimentally that is chemical and/or electrical. When using conditions in which bath and pipette solutions contain equal concentrations of the major permeant ion, there is no chemical driving force. Hence, the potential gradient needs to be large enough to generate an electrical driving force. Protocols to reliably record human CatSper inward currents have been described (3, 4). Therefore, an electrical driving force of 20 mV generated from a holding potential of −80 to −100 mV, as shown by Brenker et al. (1), is neither enough to reliably assess inward CatSper currents nor to estimate "fold current increase" (figure 1 E–G and figure 2 C–E of ref. 1). Moreover, it is misleading to show absolute currents (pA) and not cell size-based current densities (pA/pF), since sperm cells vary in size.

When performing calcium imaging with spermatozoa, it is important to specify which cellular compartment was evaluated. Changes in intracellular calcium occur in the sperm head, the midpiece, and the principal piece (PP), where CatSper resides. If it is of interest to study CatSper-mediated calcium influx into spermatozoa, individual PPs as regions of interest must be analyzed (Fig. 1). Based on previous reports by the same group (5), we assume that calcium imaging was done in a cuvette yielding population-based results. Since sperm heads contain much more calcium than tails (Fig. 1*A*), strong signals from the head can mask fluorescence changes in the tail. By analyzing fluorescence changes specifically in the PP, we can support our published results (2). Precisely, we observed a dose-dependent inhibition of progesterone-evoked calcium influx into the PP via CatSper by pristimerin (Fig. 1 *C* and *D*).

Figure 1 of ref. 1 shows calcium imaging results of cells from a CatSper-deficient patient with deafness-infertility syndrome. We assume that the individual described is phenotypically similar to the individuals described by Smith et al. (6) and Avidan et al. (7). Generally, less than 10% of such patients' barely motile spermatozoa possess tails (figure 1C of ref. 6), making it impossible to separate them from somatic cells. Thus, it is impractical to analyze a sample that mainly consists of somatic cells and tail-less spermatozoa by population-based calcium imaging to demonstrate CatSper-related effects.

In summary, we stand by our data 100% (2), and conclude that the discrepancies reported by Brenker et al. (1) are due to different and uncontrolled conditions used to perform the experiments.

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The authors declare no conflict of interest.

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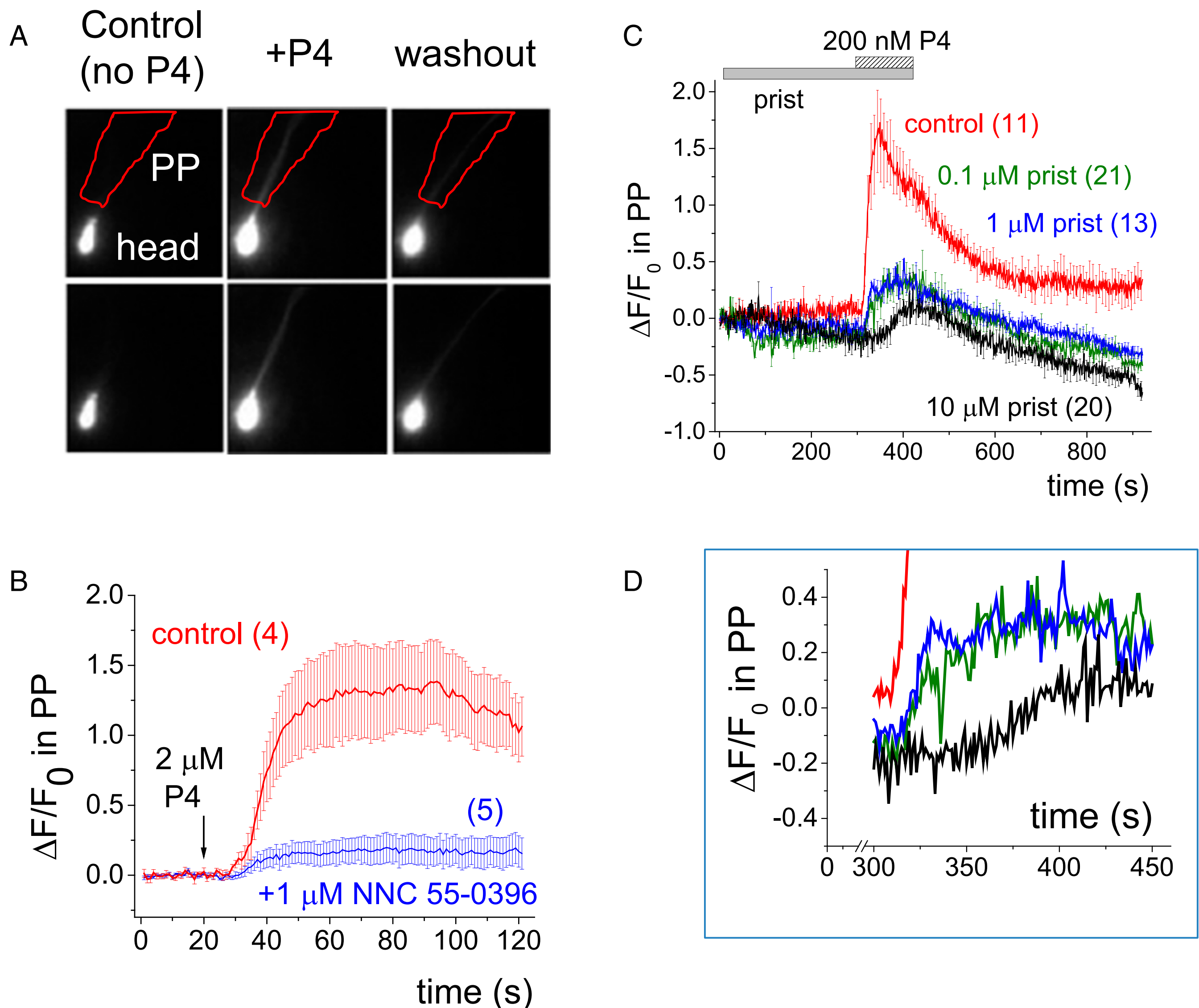

Fig. 1. **Pristimerin diminishes the progesterone-induced calcium influx via CatSper. (*A*) Representative images of a Fluo-4–loaded human spermatozoon to collect fluorescence intensities in the PP. Red outline shows the region of interest around the principal piece. (Magnification: 800×.) (*B*) Progesterone (P4) evokes calcium influx into the PP via CatSper. Noncapacitated spermatozoa were isolated as described (2), and stimulated with P4 ± CatSper inhibitor NNC 55-0396. (*C*) Spermatozoa were stimulated with P4 alone (control) or with indicated concentrations of pristimerin (prist). (*D*) Inset of *C* shows dose-dependent activation delays in the presence of pristimerin. Spermatozoa were stimulated via continuous perfusion. The number of analyzed cells from two human donors are depicted in parentheses.**

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