Fig. S1. Immunohistochemical detection of iGluR2 protein in single islet cells. *A*: α cells identified using glucagon-specific antibody express the iGluR2 subtype of AMPA receptor. 24 out of 26 identified α cells were positive to iGluR2 antibody (Santa Cruz, sc-7611). *B*: a glucagon-positive α cell was stained with another iGluR2-specific antibody directly labeled with Alexa-488 dye (Alomone, GluR2-ATTO_488). 15 out of 17 identified α cells expressed iGluR2 immunoreactivity. *C*: an insulin-positive β cell, 20 out of identified 23 β cells expressed iGluR2. *D*: a somatostatin-positive δ cell. For this staining we used FITC-labeled secondary antibody for somatostatin and TRITC-labeled secondary antibody for iGluR2. For consistency with other labelings, the color coding was switched. Only 3 out of 11 δ cells showed a weak iGluR2 signal. Scale bars indicate 5 μm. Antibodies used were as follows. *A*: mouse anti-glucagon (1:100 dilution)/goat anti-mouse TRITC (1:500) and rabbit anti-iGluR2 (1:100)/goat anti-rabbit FITC (1:200). *B*: mouse anti-glucagon (1:100)/goat anti-mouse TRITC (1:500) and rabbit anti-iGluR2 labeled with Alexa-488 (1:30). *C*: guinea pig anti-insulin (1:100 dilution)/goat anti-guinea pig FITC (1:500) and rabbit anti-iGluR2 (1:100)/goat anti-rabbit TRITC (1:200). *D*: mouse anti-somatostatin (1:100)/goat anti-mouse TRITC (1:500) and rabbit anti-iGluR2 (1:100)/goat anti-rabbit FITC (1:200).

Fig. S2. Activation of islet iGluR by different agonists. *A*: time courses of inward current activated by 100-ms pulses of 1 mmol/l L-glutamate, AMPA, and kainate at –60 mV. All records were obtained from the same lifted whole cell in $Na^+$-rich external solution. *B-D*: concentration dependence of peak currents activated by L-glutamate ($n$ = 8 - 10), AMPA ($n$ = 5 - 8) and kainate ($n$ = 9 - 12) at –60 mV. Peak current amplitudes were normalized to the value obtained with 1 mmol/l of each agonist and were fitted with the Hill equation (Eq. 1). The Hill coefficients for all agonists were nearly unity: 1.37 for L-glutamate, 1.10 for AMPA, 0.93 for AMPA peak currents (filled circles), and 0.98 for AMPA steady-state currents (empty circles).

Fig. S3. Single-channel recording of iGluRs. Top three traces were recorded from an excised outside-out patch upon 10 mM glutamate application at –60 mV. The ensemble average obtained from 95 traces from the same patch showed a desensitization time constant of 6.0 ms (broken line). Single traces were filtered at 500 Hz and the traces used for the ensemble average were filtered at 1 kHz. In some traces single-channel opening at the end of desensitization was observed as marked with an arrow.

Fig. S4. Current-voltage relations of iGluRs. *A-C*: time courses of currents evoked by 100 ms pulses of 1 mmol/l L-glutamate, AMPA, and kainate. Membrane potential was varied between –80 mV and 80 mV in 20 mV steps. All traces were obtained from the same cell. *D-F*: current-voltage relations of

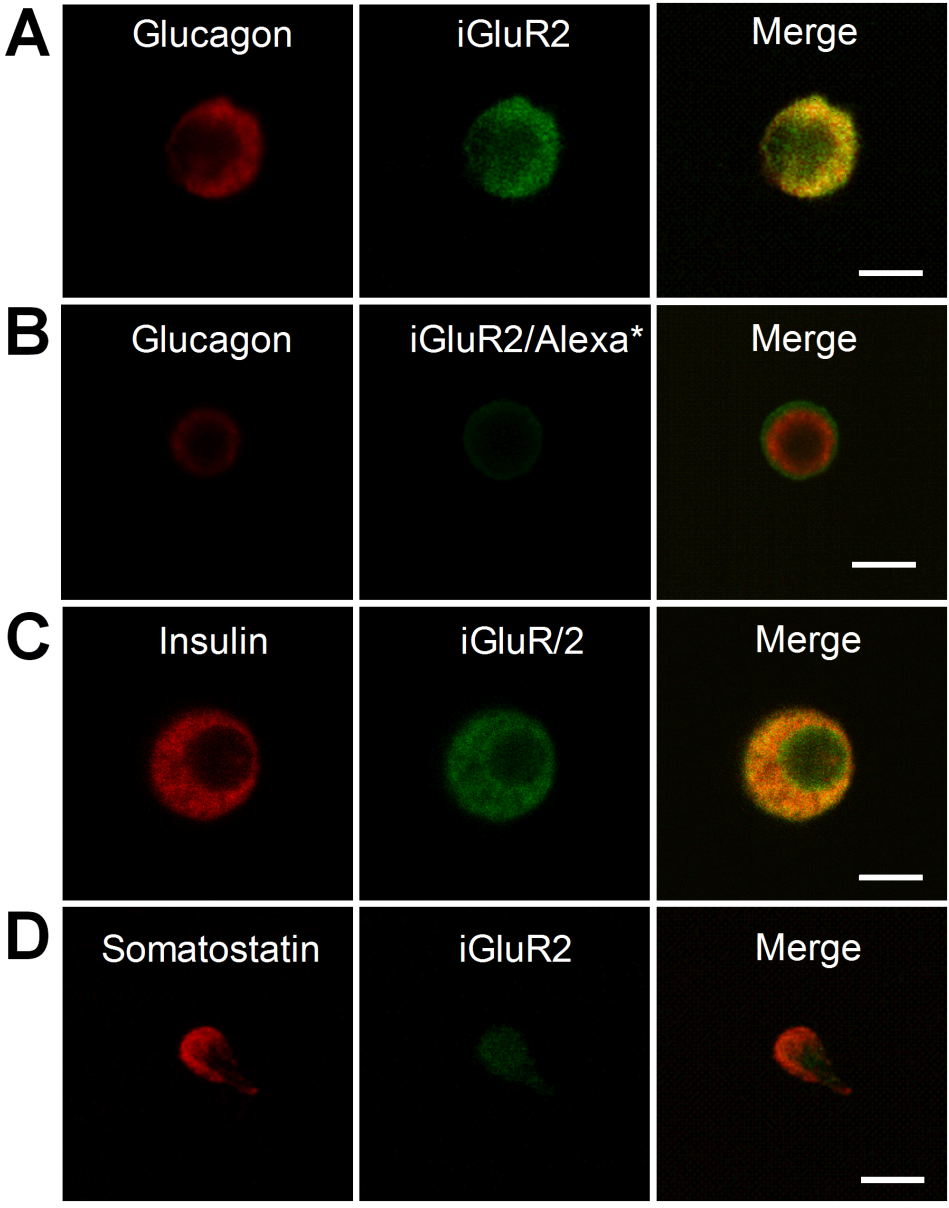
the agonist-evoked peak currents. Data points were fitted with second-order polynomial curves. Reversal potentials for L-glutamate, AMPA and kainate were 3.6, 8.2, and 2.1 mV, respectively, in this single experiment. To describe the curvature of the *I-V* relations quantitatively, a rectification index (RI) was defined as the ratio of the whole-cell slope conductance at +40 mV and –40 mV. RI values of 1, >1, <1 indicate linear, outwardly rectifying, and inwardly rectifying *I-V* relations, respectively. The RI was 0.9 ± 0.04 ($n$ = 5) for L-glutamate, 0.9 ± 0.1 ($n$ = 7) for AMPA, and 1.8 ± 2.2 ($n$ = 9) for kainate.

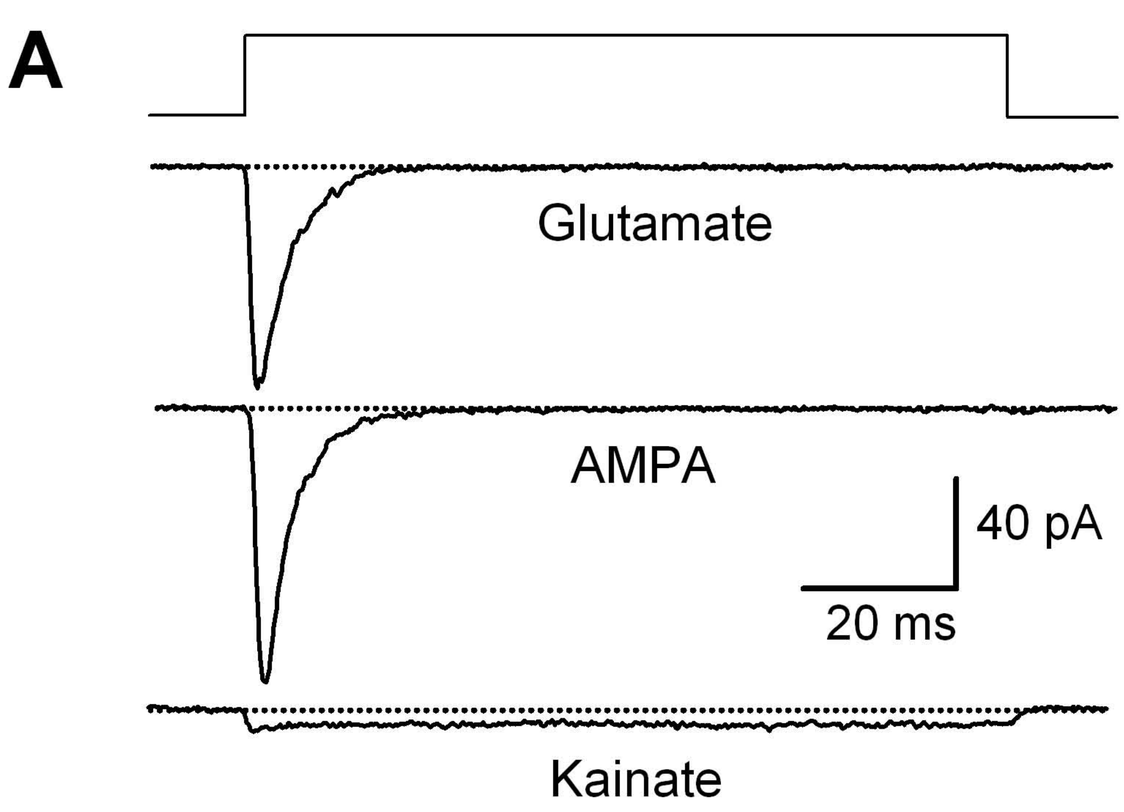
Fig. S5. $Ca^{2+}$ permeability of iGluRs. *A*: time courses of L-glutamate-evoked currents with 135 mmol/l $Na^{+}$-rich external solution and evoked by 100 ms pulses of 1 mmol/l L-glutamate. Membrane potentials were varied between –80 and 80 mV in 10 mV steps but only 20 mV intervals were displayed for clarity. *B*: current records from the same cell as *A* in 100 mmol/l $Ca^{2+}$- rich external solution. *C*: *I-V* relations of peak currents (●, $Na^{+}$-rich and ○, $Ca^{2+}$-rich solutions). Measured reversal potentials were –5.4 mV and –45.1 mV, respectively. *D*: rectification index (RI) plotted against $Ca^{2+}$ permeability ($n$ = 10). A scatter plot of the $Ca^{2+}$ permeability against the RI calculated in $Na^{+}$-rich solution showed that islet iGluRs have a mean RI near 1.0, indicating a linear *I-V* relation and relatively low $Ca^{2+}$ permeability.

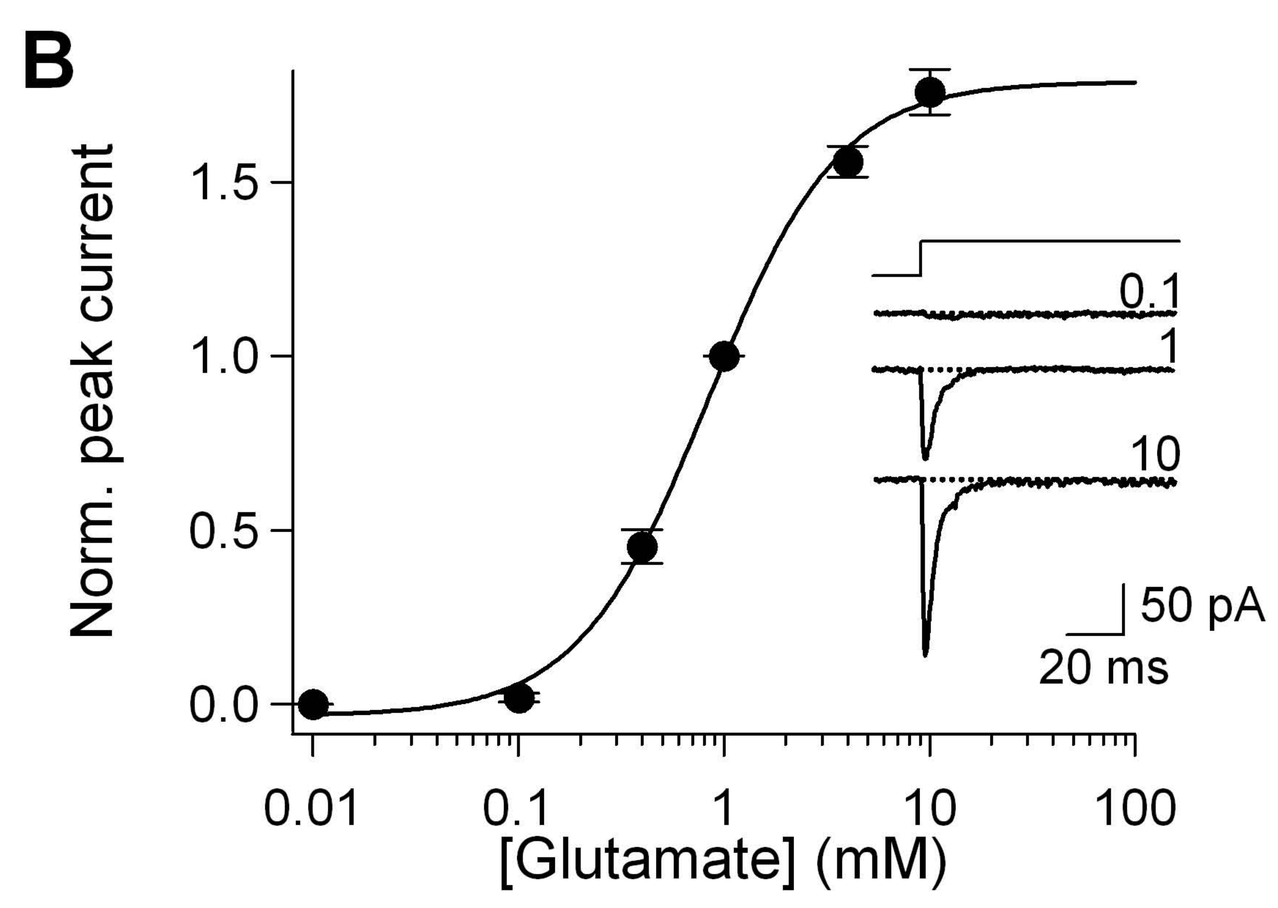
Fig. S6. *A*: Cytoplasmic accumulation of serotonin (5-hydroxytryptamine) was indirectly assessed using its fluorescent analog 5,7-dyhydroxytryptamine (5,7-DHT, shown as inset with an additional hydroxyl group marked). Islet cells were incubated with 1 mM 5,7-DHT and 1 mM ascorbic acid to reduce spontaneous oxidation of 5,7-DHT for different times. 5,7-DHT fluorescence was measured at 410 nm with excitation at 360 nm. Open circles are the average 5,7-DHT fluorescence measured from all islet cells measured and closed triangles from cells smaller than 7 μm in diameter. After 6 hr-incubation, cells were treated with 1 μM of bafilomycin A1 (Calbiochem) for 10 min and 5,7-DHT fluorescence was measured (red symbols). Increase of fluorescence upon bafilomycin A1 may suggest cytoplasmic release of 5,7-DHT from secretory granules where the dye is not fluorescent due to low

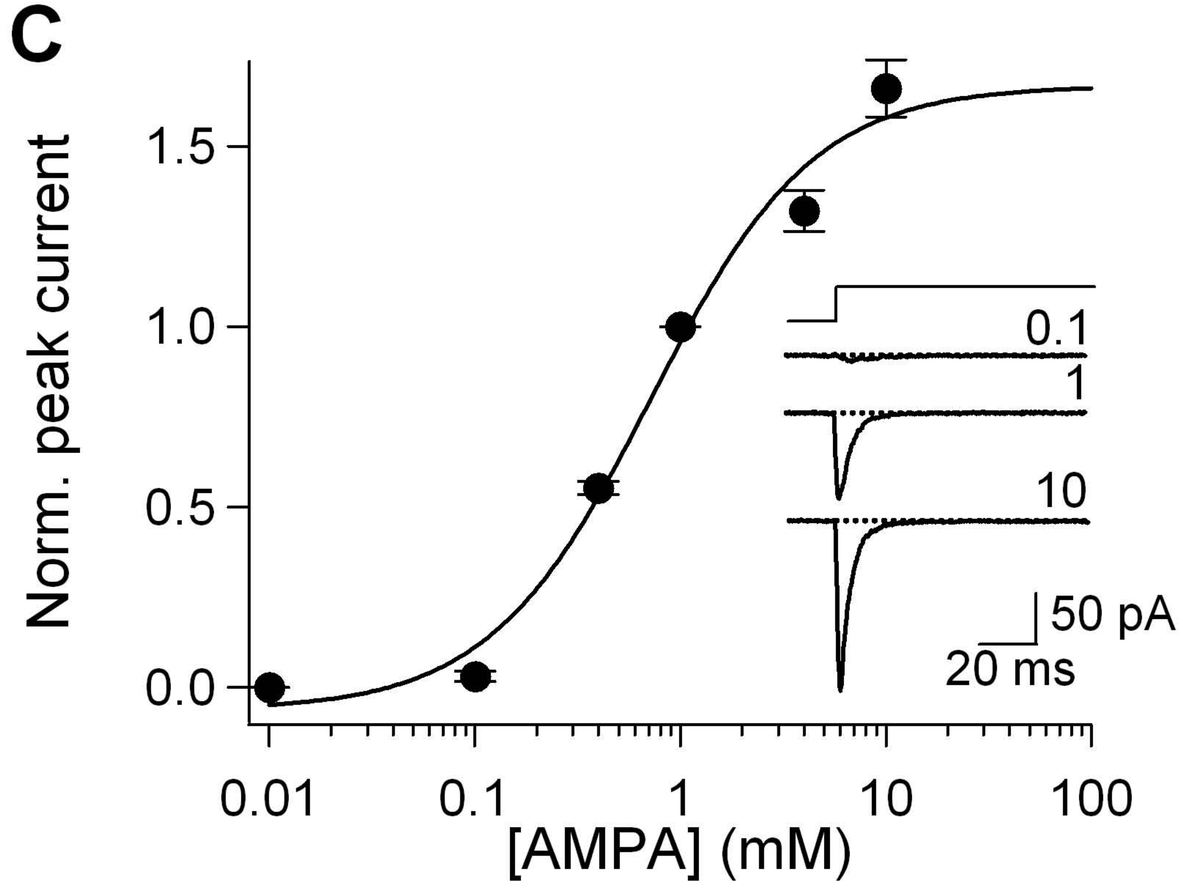
pH. Alternatively 5,7-DHT became fluorescent after collapse of proton gradient and increase of intragranular pH. Fluorescence and cell size were determined using a high magnification lens (100 X). *B:* Immunocytochemical detection of vesicular monoamine transporter (VMAT2, Millipore, AB1767) proteins in single islet cells. α cells identified using glucagon-specific antibody were positive to anti-VMAT2 antibody (15 out of 15 identified α cells). β cells expressed VMAT2 proteins (17 out of 17 identified β cells). Scale bars indicate 5 μm. Antibodies used were as follows. *B*: mouse anti-glucagon (1:100 dilution)/donkey anti-mouse IgG-FITC (1:100) and rabbit anti-rat VMAT2 (1:50)/goat anti-rabbit IgG-TRITC (1:1000). Guinea pig anti-insulin (1:500 dilution)/goat anti-guinea pig Alexa488 (1:1000) and rabbit anti-rat VMAT2 (1:50)/goat anti-rabbit TRITC (1:1000).

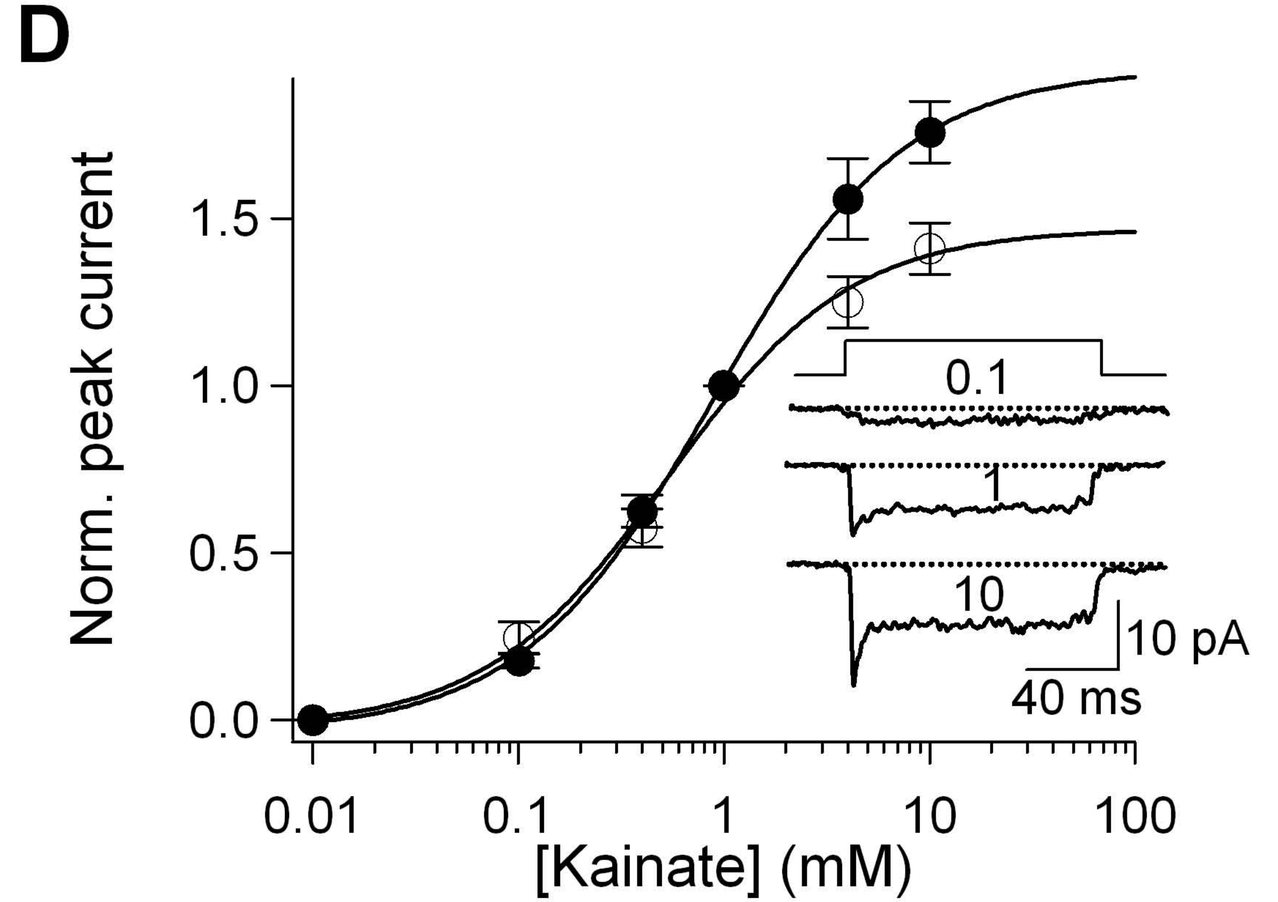
Fig. S7. Three types of $[Ca^{2+}]_i$ responses to kainate and glucose stimulation. Single islet cells are incubated with 3.3 mmol/l glucose initially and then challenged successively with 0.5 mmol/l kainate (KA) for 10 s to test for iGluRs, with 16.7 mmol/l glucose for 5 min, with kainate again, and finally with 135 mmol/l $K^+$ external solution for 10 s to verify responsiveness to depolarization.

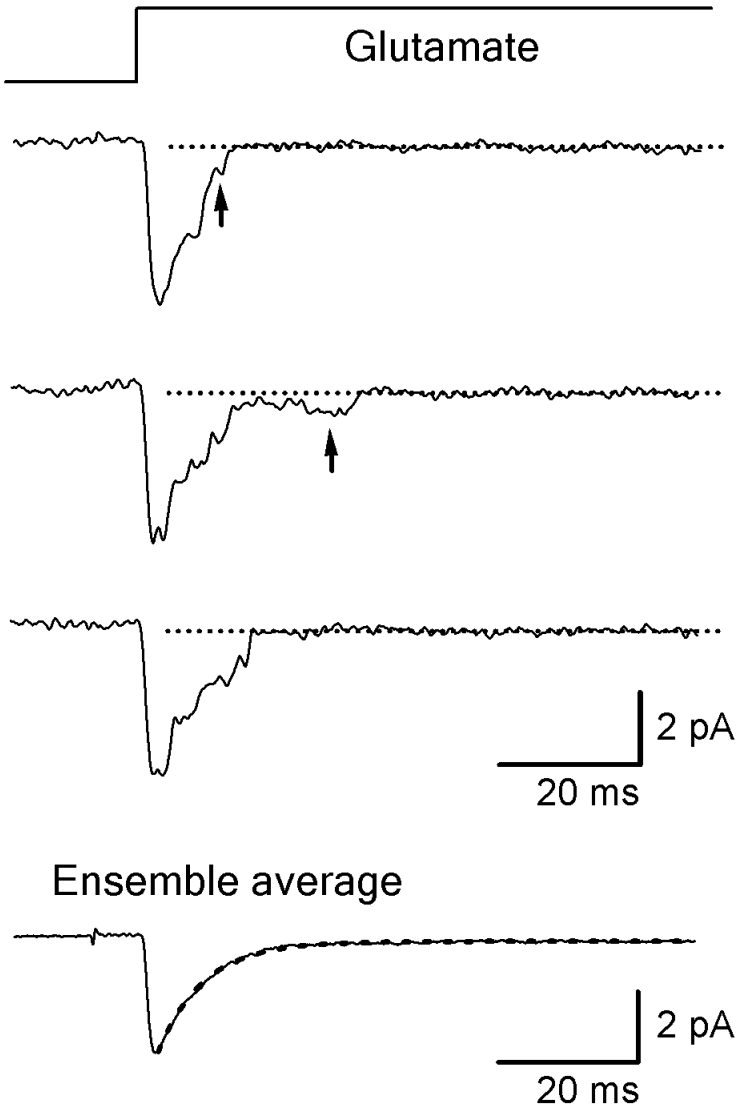

A
Glucagon
iGluR2
Merge
B
Glucagon
iGluR2/Alexa*
Merge
C
Insulin
iGluR/2
Merge
D
Somatostatin
iGluR2
Merge

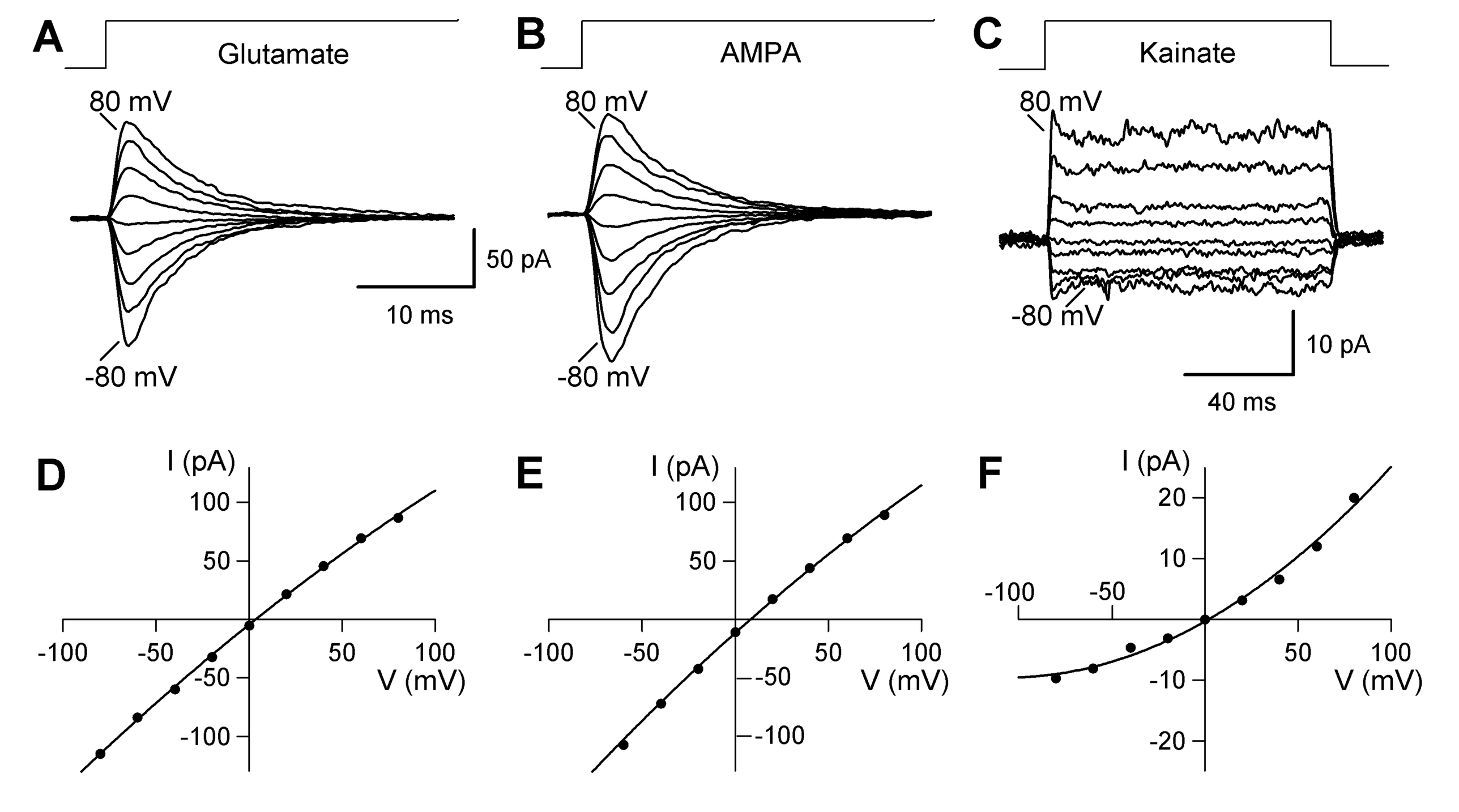

A
Glutamate
AMPA
40 pA
20 ms
Kainate


B
Norm. peak current
1.5
1.0
0.5
0.0
0.1
1
10
50 pA
20 ms
0.01
0.1
1
10
100
[Glutamate] (mM)


C
Norm. peak current
1.5
1.0
0.5
0.0
0.1
1
10
50 pA
20 ms
0.01
0.1
1
10
100
[AMPA] (mM)


D
Norm. peak current
1.5
1.0
0.5
0.0
0.1
1
10
10 pA
40 ms
0.01
0.1
1
10
100
[Kainate] (mM)


Glutamate
2 pA
20 ms
Ensemble average
2 pA
20 ms


A
Glutamate
80 mV
-80 mV
50 pA
10 ms
B
AMPA
80 mV
-80 mV
C
Kainate
80 mV
-80 mV
10 pA
40 ms
D
I (pA)
100
50
-50
-100
-100
-50
50
100
V (mV)
E
I (pA)
100
50
-50
-100
-100
-50
50
100
V (mV)
F
I (pA)
20
10
-10
-20
-100
-50
50
100
V (mV)

A

Glutamate + 2.5 Ca

80 mV

-80 mV

50 pA

10 ms

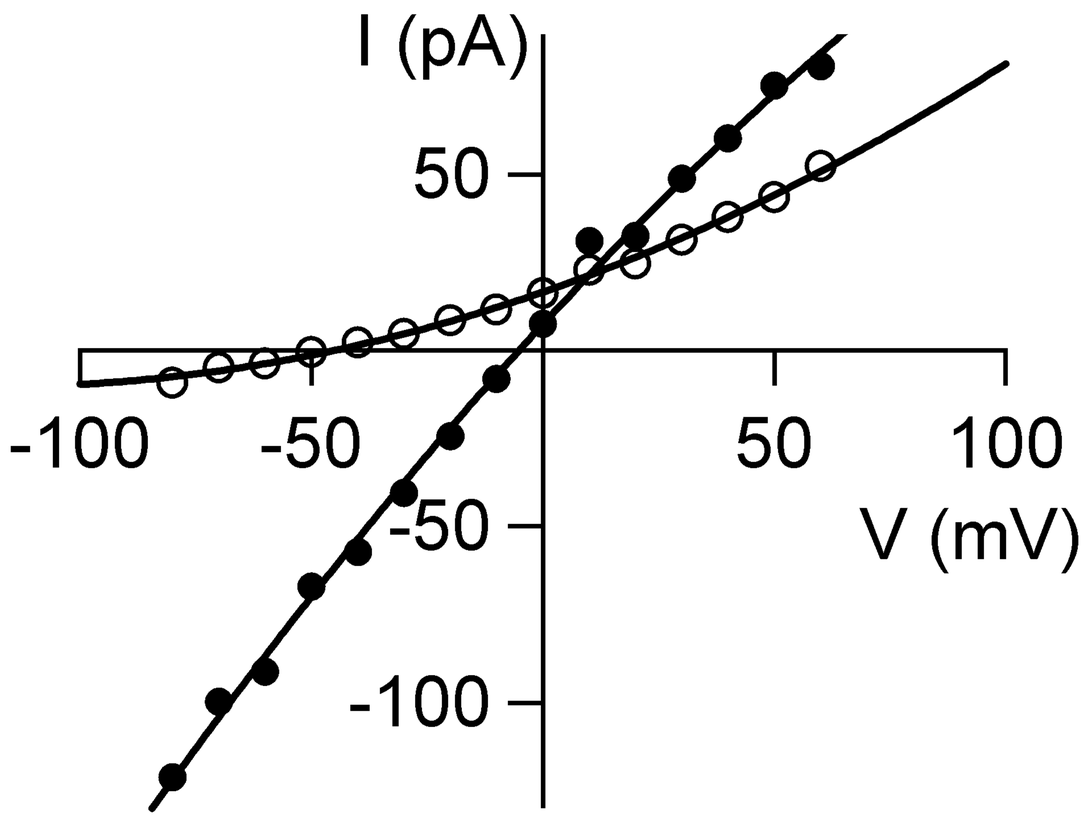
B

Glutamate + 100 Ca

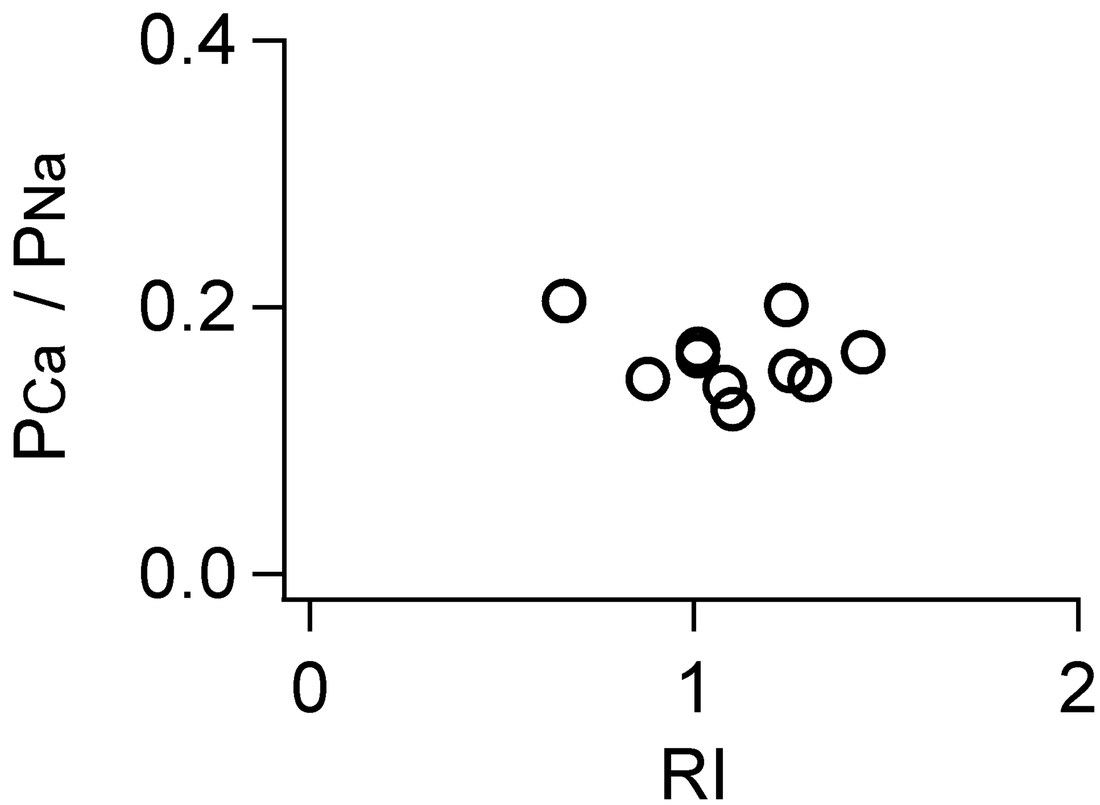
80 mV

-80 mV

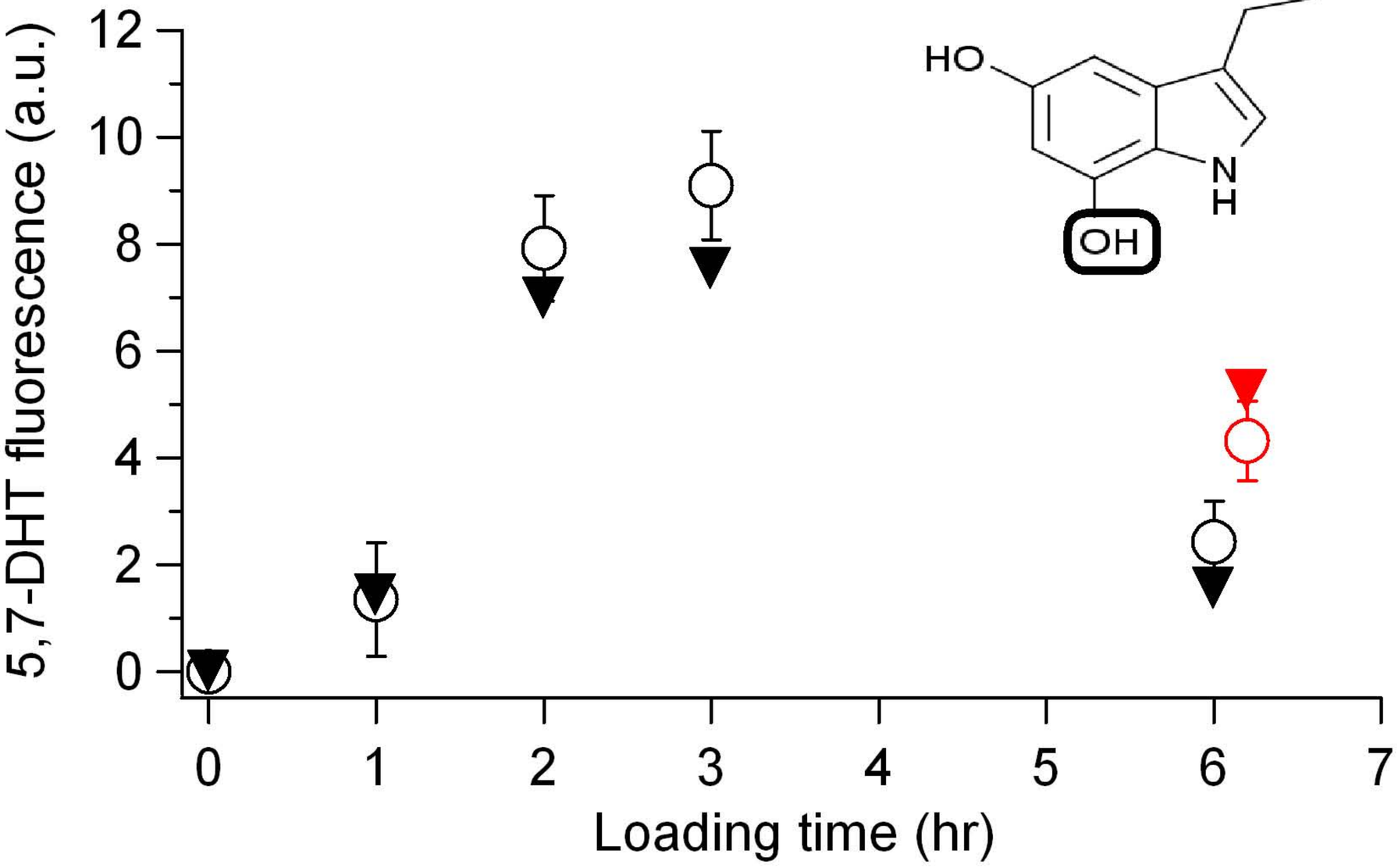
C



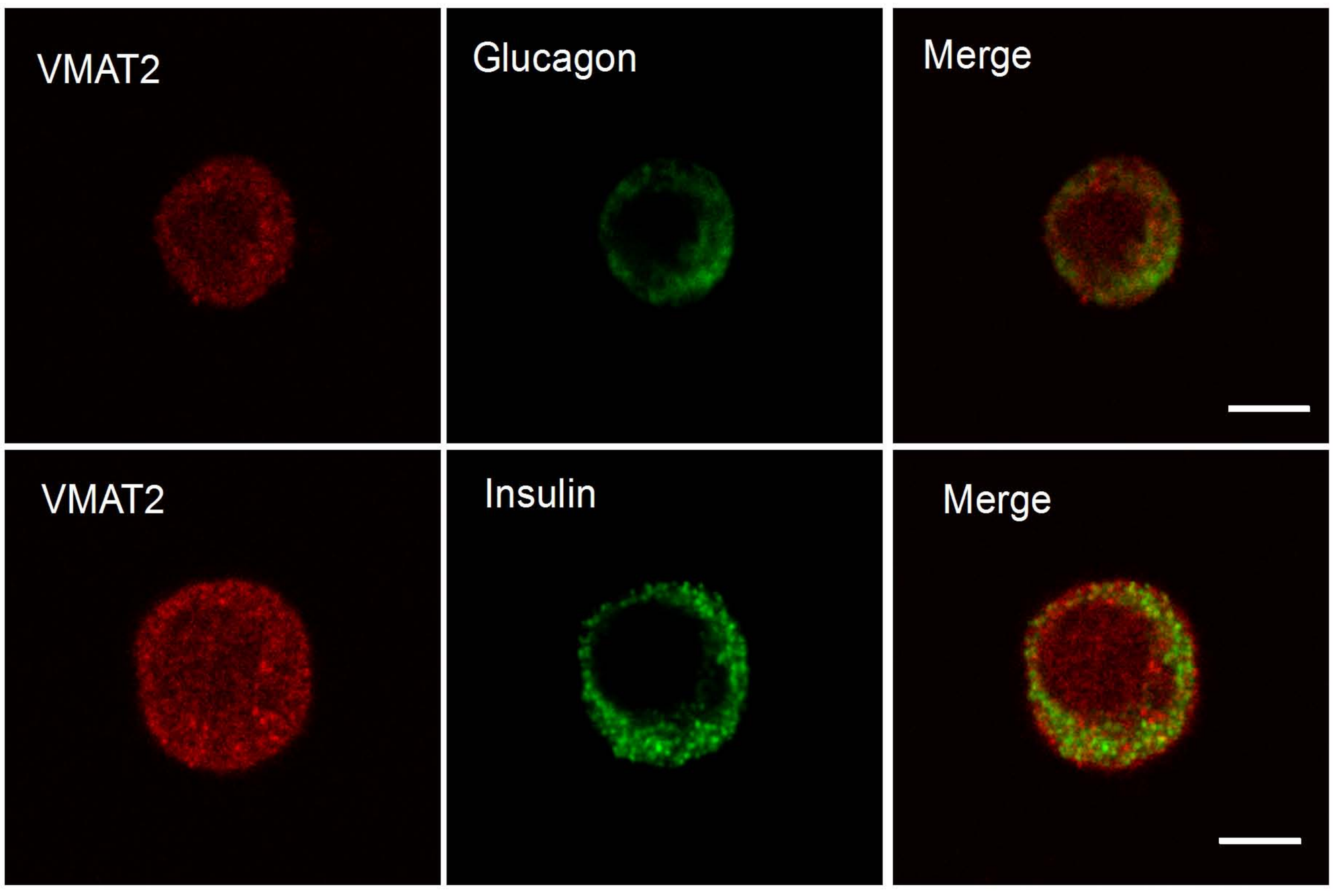

D



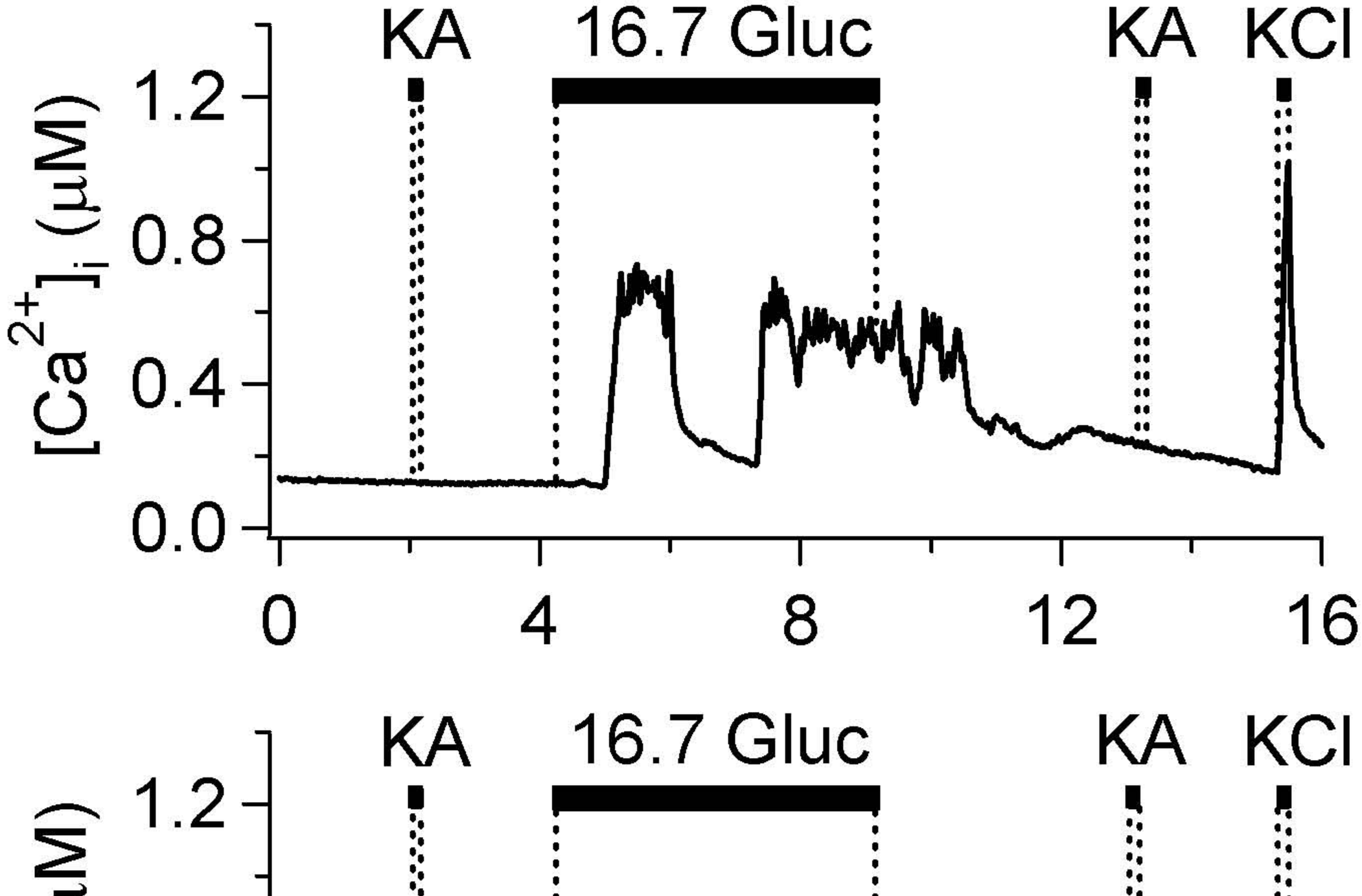
**A**


5,7-DHT fluorescence (a.u.)
0
2
4
6
8
10
12
0
1
2
3
4
5
6
7
Loading time (hr)
NH2
HO
N
H
OH

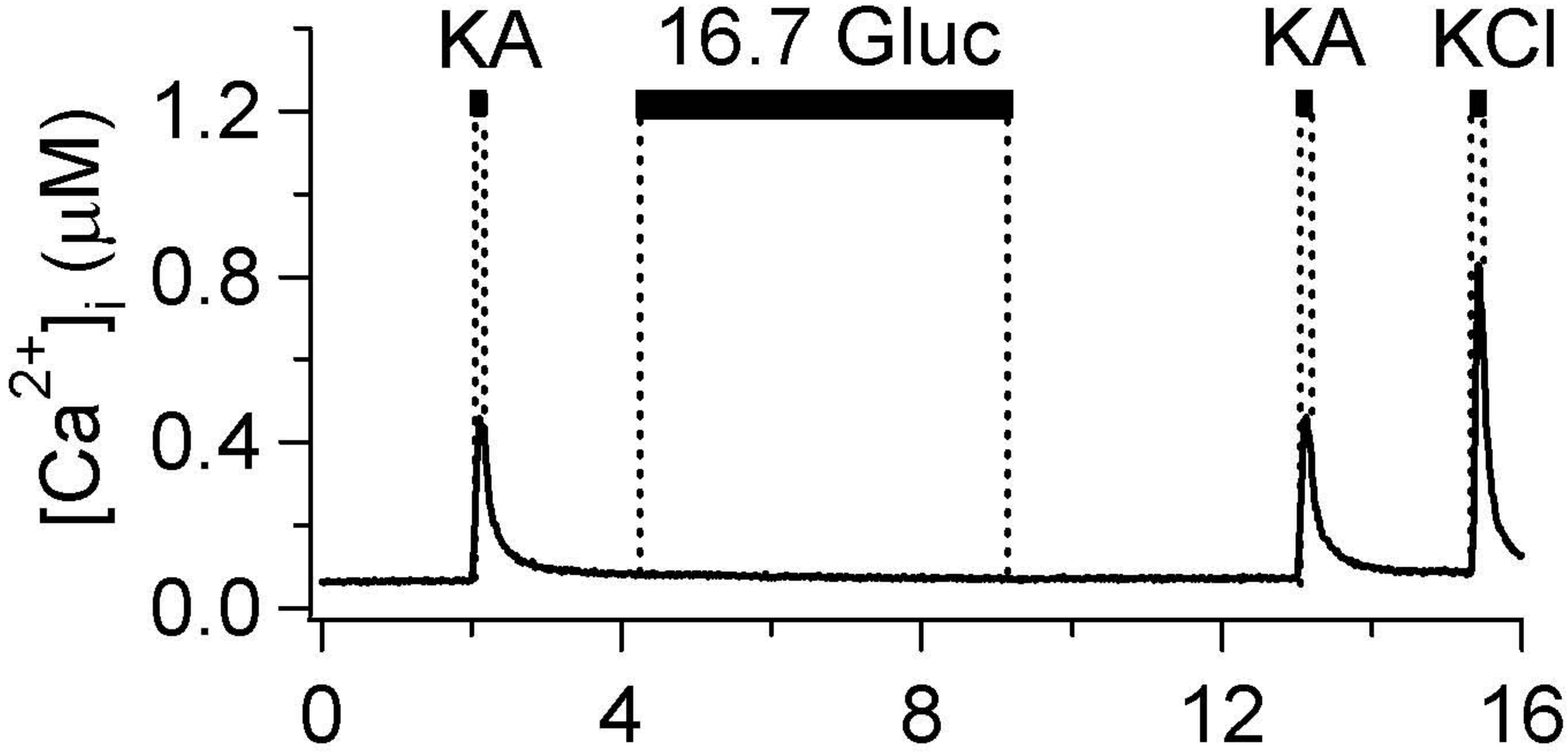
**B**


VMAT2
Glucagon
Merge
VMAT2
Insulin
Merge

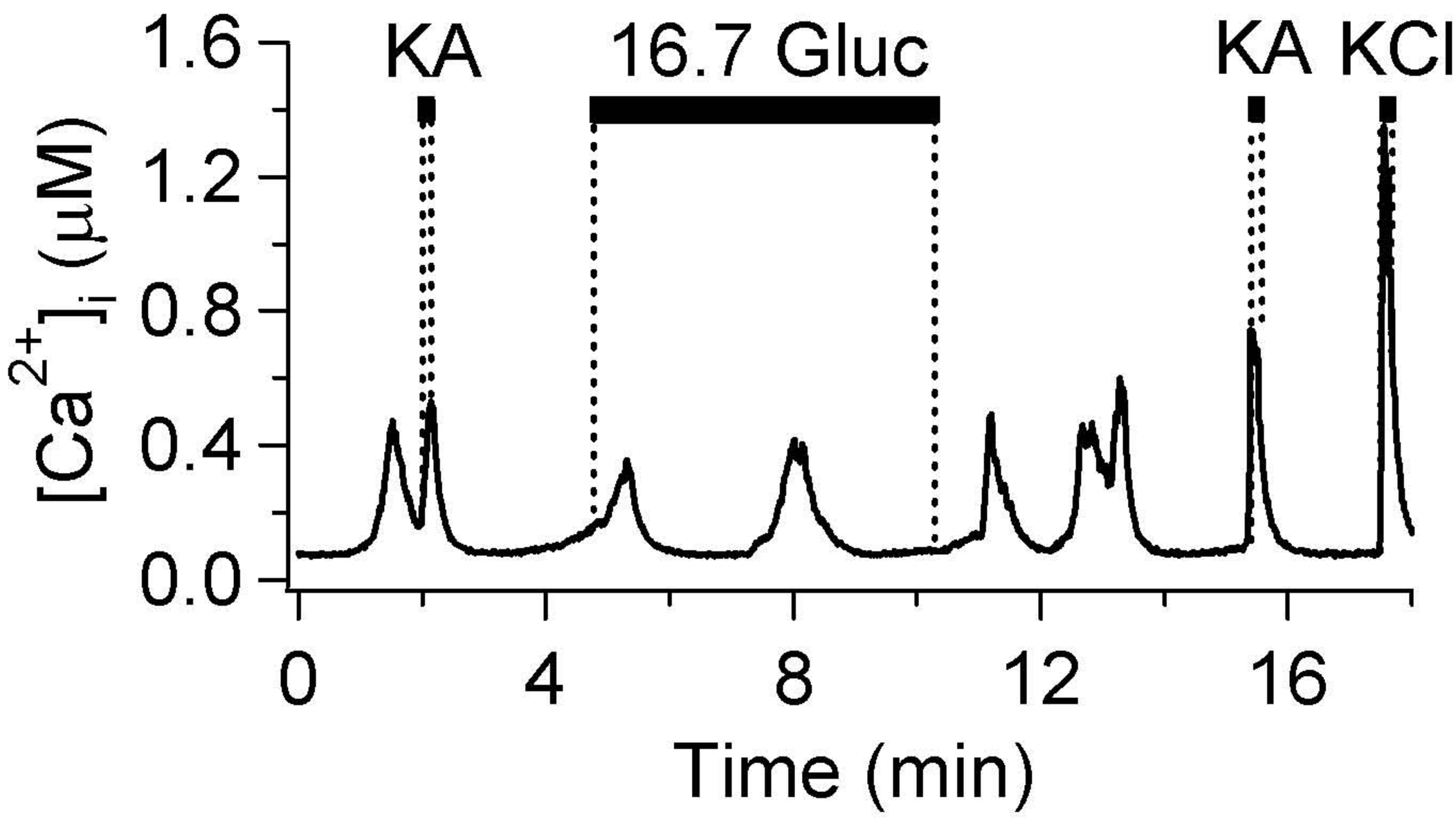
**A**


KA
16.7 Gluc
KA
KCl
$[Ca^{2+}]_i$ (μM)
1.2
0.8
0.4
0.0
0
4
8
12
16

**B**


KA
16.7 Gluc
KA
KCl
$[Ca^{2+}]_i$ (μM)
1.2
0.8
0.4
0.0
0
4
8
12
16

**C**


KA
16.7 Gluc
KA
KCl
$[Ca^{2+}]_i$ (μM)
1.6
1.2
0.8
0.4
0.0
0
4
8
12
16
Time (min)