Caption:   
Intracellular transport of endocytosed ligands. Cultures of LSEC were pulsed with both TRITC-FSA and FITC-AGG for 1 h at 4°C. Chasing was performed after removal of unbound ligand by washing, and transferring of the cultures to 37°C. The cultures were fixed after chase periods of 10 min or 2 h and examined in fluorescence microscope. At 10 min (A) all TRITC-FSA co-localized with FITC-AGG (yellow colour indicates co-localization) in large ring-shaped vesicles (large arrowheads), and some vesicles with only FITC-AGG were observed (small arrowheads). After 2 h (B), co-localization of FITC-AGG and TRITC-FSA in large vesicles (arrows) was observed together with big vesicles with only FITC-AGG (large arrowheads) and small vesicles with only TRITC-FSA (small arrowheads). Controls show a more perinuclear appearance of TRITC-FSA when pulsed and chased for 2 h alone (C). In other experiments, TRITC-FSA was injected intravenously 1.5 h before isolation of the cells. Following an additional 6.5 h of cultivation at 37°C cultures of LSEC were pulsed with FITC-FSA (D-E), FITC-collagen (F-G), or FITC-mannan (H-I) for 1 h at 4°C. Following a 10 min chase, the FITC-ligands were observed to appear in small vesicles (arrowheads), and did not co-localize with TRITC-FSA (D, F and H). After 2 h, the FITC-ligands were transported to perinuclear compartments that co-localized almost completely with TRITC-FSA (small arrows in E, G and I). Other cultures of LSEC were pulsed for 10 min at 37°C with FITC-bHA. Following a 20 min chase, the FITC-bHA was observed in vesicles distributed all over the cell (arrowheads in J), and a similar appearance was observed after 2 h (arrowheads in K). Occasionally, cells that did not take up TRITC-FSA in vivo, but endocytosed FITC-ligands in vitro (big arrow in I), were observed. Scale bars: 10 μm.

Question: How long were the LSEC cultures pulsed with TRITC-FSA and FITC-AGG at 4°C?   
   
A: 1 h.   
B: 10 min.   
C: 2 h.   
D: 4°C.

Answer: A: 1 h.