Beckman ultracentrifuge and a SW 41 rotor for 16~20 h at 4°C. Following ultracentrifugation, twelve 1-ml fractions were collected from the bottom of the gradient tube using a peristaltic pump (RAININ). FLAG-mDOR and caveolin-1 in each fraction were examined by Western blot as mentioned in MATERIALS AND METHODS. **(B) Co-immunoprecipitation of $G_{\alpha i}$ proteins and caveiolin-1 in CHO-FLAG-mDOR cells was reduced by MCD treatment**. Cells were treated with vehicle or 2% MCD and harvested as described under Materials and Methods. Cells were solubilized with 2% Triton X-100 and then centrifuged at 100,000 x *g*. The supernatant was incubated with rabbit anti-$G_{\alpha i\text{-}3}$ antibody, which recognizes all $G_{\alpha i}$ proteins, or normal rabbit serum and then with Protein A/G PLUS-Agarose. Immunoprecipitated materials were dissolved in 2X Laemmli loading buffer, resolved in 10% SDS-PAGE and transferred to Immobilon-P PVDF membranes. Immunoblotting was performed with a monoclonal antibody against caveolin-1 (top panel). One tenth of the supernatant was resolved and blotted as well. The membranes were then stripped and blotted again with the rabbit anti-$G_{\alpha i\text{-}3}$ antibody (bottom panel). The figures represent one of the three experiments performed with similar results.

Fig. S1. Discontinuous and continuous sucrose density gradients and collection of 12 1-ml fractions.

Fig. S2. About 70% of opioid receptors in membranes of the rat caudate putamen (CPu) locate in rafts fractions with a 5-20% sucrose density. Rat CPu membranes were sonicated in 0.5 M sodium carbonate buffer (pH 11) and then fractionated through a continuous sucrose gradient (**Fractions 1~8: 5%~35%; Fractions 9~12: 45%**) by ultracentrifugation as described under Materials and Methods (Fig. S1). Twelve 1-ml fractions were collected and each fraction was subjected to

(A) Determination of cholesterol contents. Data are expressed as the ratios of [cholesterol in each fraction]/ [total phospholipids].

(B) Determination of GM1 levels by a dot-blot assay with an anti-GM1antibody.

(C) [^{3}H]diprenorphine (~1nM) binding using naloxone (10 μM) to define nonspecific binding. Two 100-μl aliquots from each fraction were used in binding in duplicate as described in Materials and Methods. Data are expressed as % of total specific [^{3}H]diprenorphine binding.

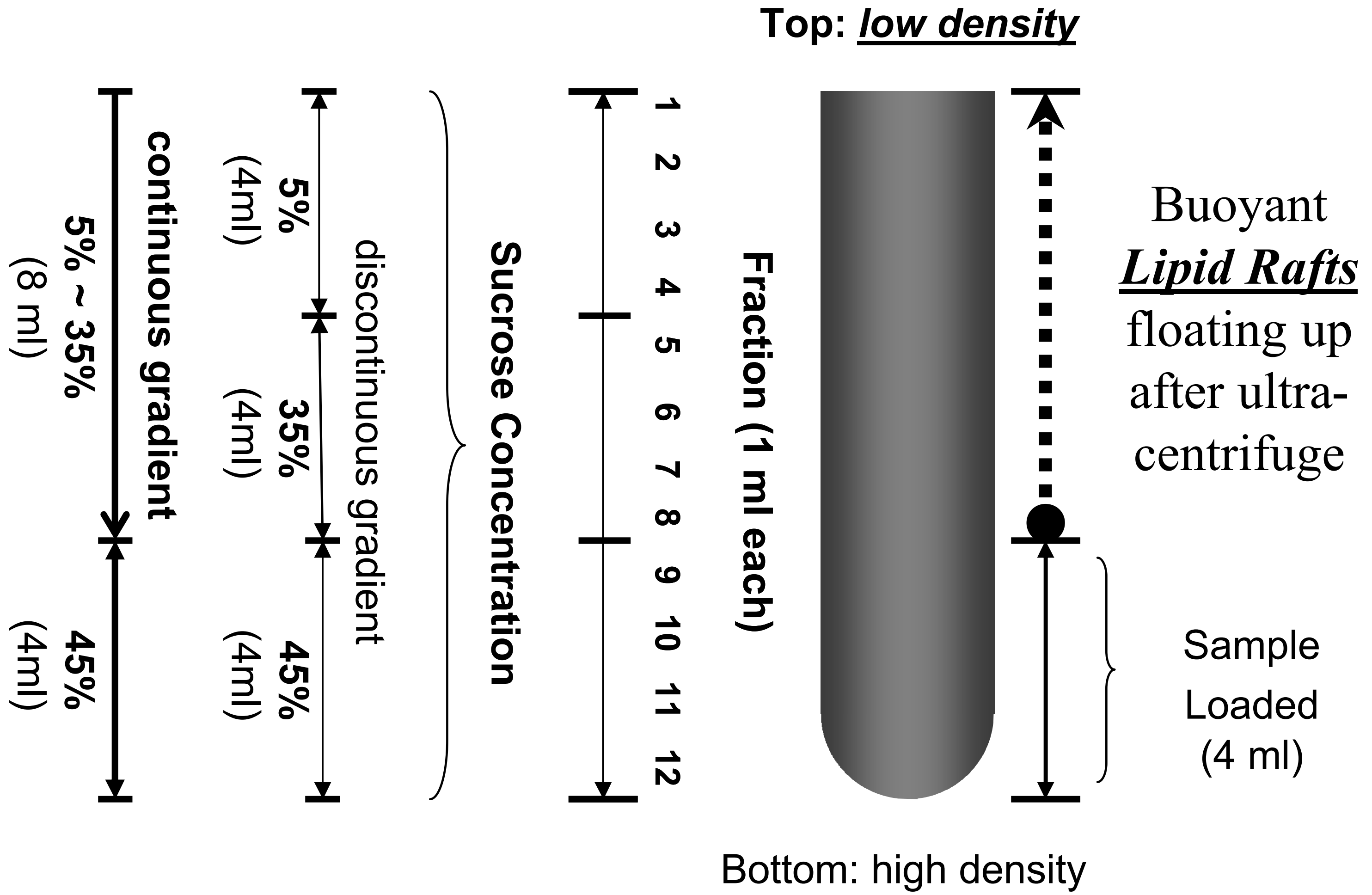
(D) SDS-PAGE, transfer to membranes and protein staining with Ponceau S.

In this set of experiments, CPu membranes prepared from 6 rats were used for fractionation. The average [^{3}H]diprenorphine specific binding in the fraction 1 was 6,801 /100 µl for rat CPu, respectively. Data in Fig. 1A and 1C are shown as mean ± s.e.m. of three independent experiments. Figures 1B and 1D represent one of the three experiments performed with similar results.

Fig. S3. Full agonists moved some DOR out of lipid rafts but partial or inverse agonists did not. NG108-15 cells were left untreated or incubated in the media in the presence of **(A)**1 µM DPDPE, deltorphin II or etorphine **(B)** 1 µM levorphanol, ICI174864 or 10 µM morphine for 30 min at 37°C. See Fig. 4 legend for more information. Data shown in (A) represents one of the two experiments performed with similar results. Data in (B) are shown as mean ± s.e.m. of at least three independent experiments.

Fig. S4. Effect of etorphine to shift DOR out of lipid rafts was not affected by pertussis toxin (PTX) pretreatment. NG108-15 cells were treated with 100 ng/ ml PTX for 24 hrs, and were then incubated in the absence or presence of 1 µM etorphine for 30 min at 37°C. See Fig. 4 legend for more information. Data shown represents one of the two experiments performed with similar results.

Fig.S1



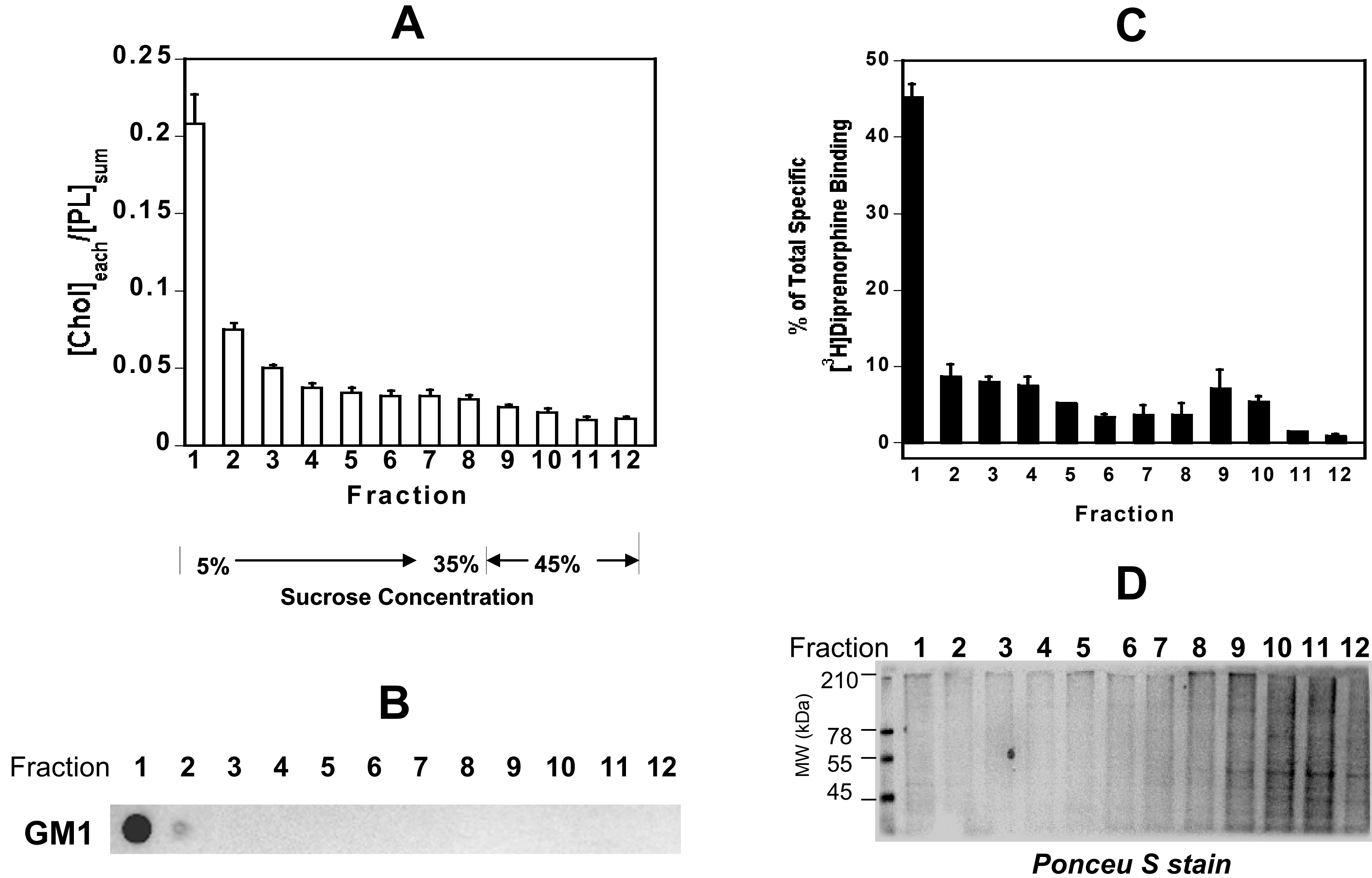


Fig. S2
Rat Brain
Caudate Putamen (CPu)
continuous sucrose gradient
A
0.25
0.2
0.15
0.1
0.05
0
[Chol]each/[PL]sum
1 2 3 4 5 6 7 8 9 10 11 12
Fraction
5%
35%
45%
Sucrose Concentration
B
Fraction 1 2 3 4 5 6 7 8 9 10 11 12
GM1
C
50
40
30
20
10
0
% of Total Specific
[3H]Diprenorphine Binding
1 2 3 4 5 6 7 8 9 10 11 12
Fraction
D
Fraction 1 2 3 4 5 6 7 8 9 10 11 12
210
78
55
45
MW (kDa)
Ponceu S stain

Fig.S3A

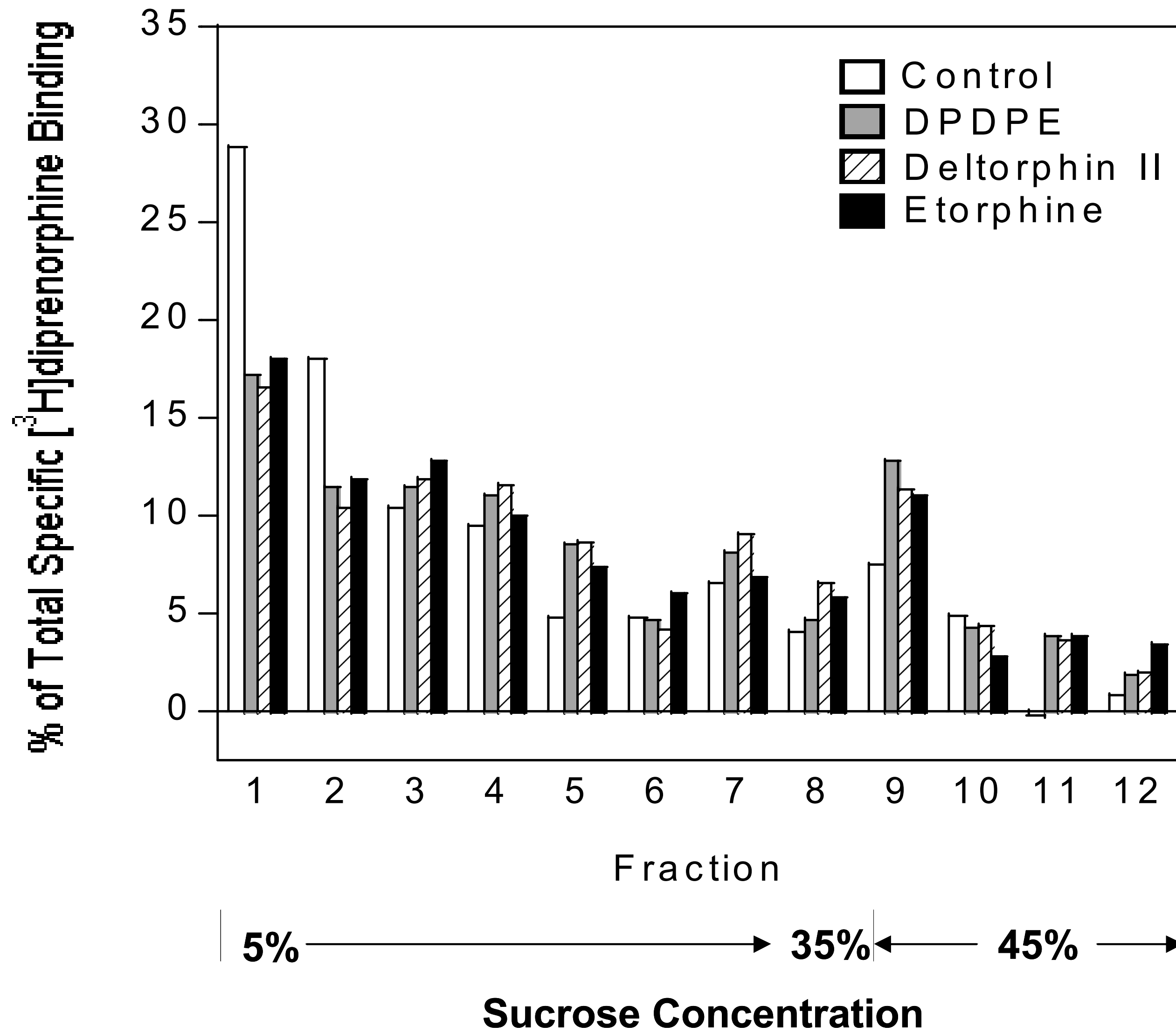


Fig.S3B

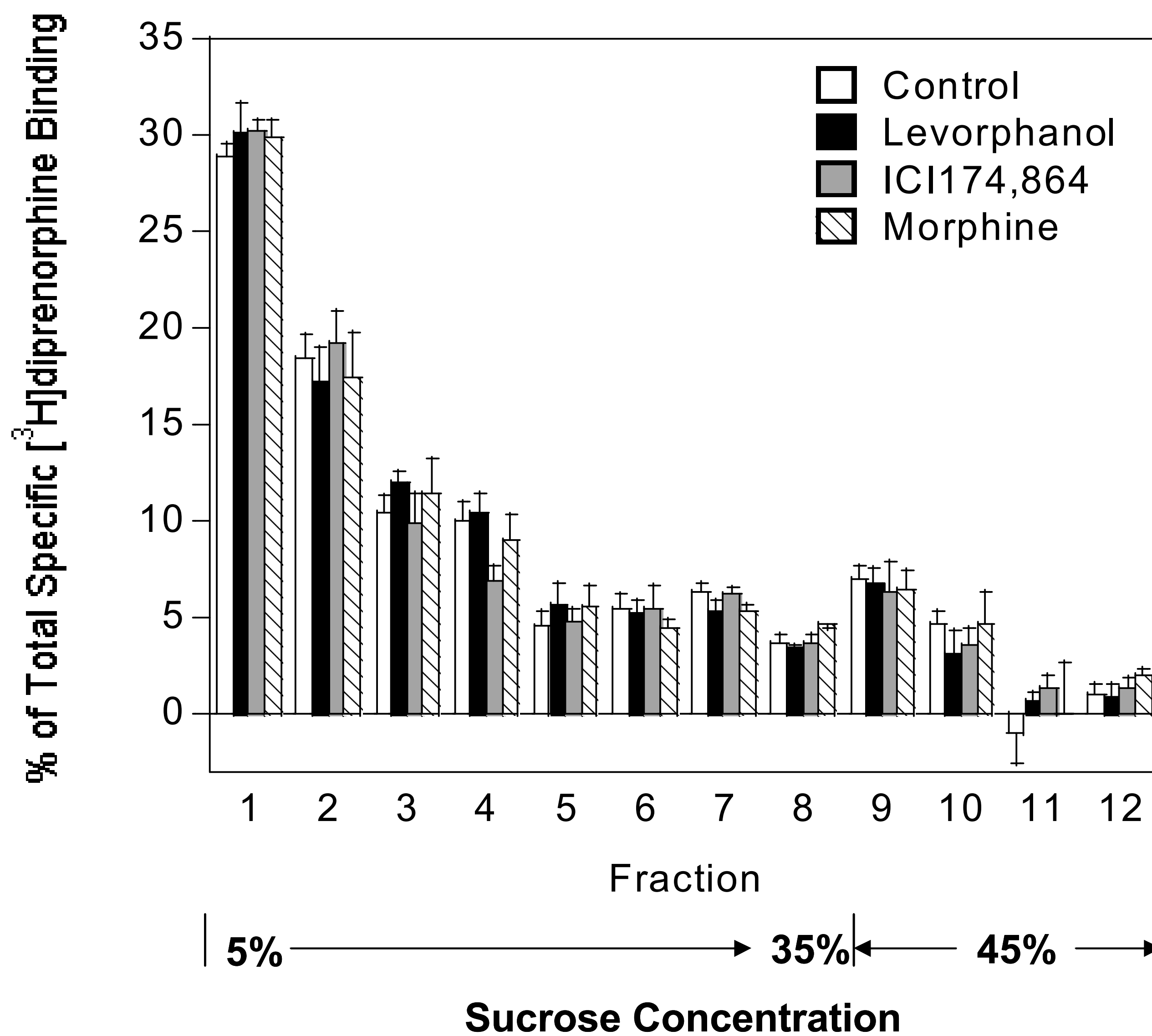


Fig. S4