Decrease and Recovery in Cell Surface Charge Induced by Anesthetics

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— We applied cell electrophoresis to detect the effect of anesthetics on membrane, and clarified the concentration-effect relationship and the term of reversibility. The cell electrophoretic mobility of rat erythrocytes decreased with time of incubation with intravenous anesthetics (thiopental or droperidol) at clinical concentrations and reached a minimum value of 25% reduction in 30 min. The effect changed gradually in the concentration range of 0.5-1000 $\mu\text{g/ml}$ of thiopental, and changed markedly with 0.3-1 $\mu\text{g/ml}$ of droperidol. Washing out of the anesthetics and subsequent incubation in serum-containing medium resulted in the complete recovery in the mobility when 5 $\mu\text{g/ml}$ thiopental or 1 $\mu\text{g/ml}$ droperidol was used. With high concentration of thiopental (100 $\mu\text{g/ml}$) or droperidol (10 $\mu\text{g/ml}$), however, unrecovered fraction of cells increased with time of treatment. The critical time for retaining the reversibility was about 2 hr. ——— membrane charge; anesthetics

It has been generally assumed that the primary action of anesthetics is on the cell membrane (Seeman 1972). Electrical stabilization of the membrane (Weidman 1955; Thesleff 1956), expansion (Seeman et al. 1969), membrane fluidization (Metcalf and Burgen 1968), membrane disordering (Spero and Roth 1970; Paterson et al. 1972; Poste et al. 1975) and change in the activity of enzymes in the membrane have been reported. The mode of action of anesthetics, however, is poorly understood. We tried to apply cell electrophoresis to detect the effect of anesthetics on the membrane and to analyze the kinetics of membrane effect. This method is to determine the net negative charge of cell surface by measuring the speed of cell movement toward the anode under a phase contrast microscope without damaging the cell (Fuhrmann and Ruhenstroth-Bauer 1965; Kojima and Yamagata 1971).

A decrease in the cell electrophoretic mobility and its recovery after x-irradiation were reported in relation to the cell survival (Sato and Kojima 1971, 1974; Sato et al. 1972). Evident decrease in the mobility of erythrocytes by clinical concentrations of intravenous anesthetics (thiopental and droperidol) was observed in this experiment. Reversibility is the prerequisite of anesthetics.

The loss of reversibility at the cellular level should be investigated in relation to the concentration of anesthetics and their duration. The time course experiment of this report revealed the critical time for retaining the reversibility at high concentration of the anesthetics.

MATERIALS AND METHODS

Erythrocytes from Wistar King A rats were freshly obtained by heart puncture before each experiment. After different durations of incubation with thiopental or droperidol in Dulbecco's phosphate buffered saline at 37°C, the erythrocytes were washed twice with the buffer solution for electrophoresis. An aliquot of the erythrocytes was transferred to the measurement of electrophoretic mobility (EPM) with a Cytopherometer (Zeiss Co.) at $25 \pm 0.5^\circ\text{C}$. The remainder was suspended in culture medium RPMI 1640 supplemented with 15% fetal calf serum, and incubated for different times in a 5% CO_2 incubator. For the measurement of EPM, each erythrocyte was allowed to move $16\ \mu\text{m}$ alternately in both directions following reversal of current under the phase contrast microscope in 67 mM phosphate buffer supplemented with 5.4% glucose (pH 7.3, ionic strength 0.167). EPM was determined from separate experiments on 30–100 cells for each point and calculated in $\mu\text{m}/\text{sec}$ per V/cm .

RESULTS

Fig. 1 shows the change in the electrophoretic mobility (EPM) of erythrocytes with time of incubation with $5\ \mu\text{g}/\text{ml}$ thiopental (clinical concentration). EPM decreased progressively with time and reached a minimum value of 25% reduction in 30 min. After different times of treatment with thiopental, cells were washed and then incubated in the culture medium containing serum. A rapid and complete recovery in the subsequent incubation in culture medium without the drug was observed for duration of treatment from 1 to 5 hr. Incubation of erythrocytes in phosphate buffered saline without the anesthetics resulted in no change in EPM.

Fig. 1. Change in the mean electrophoretic mobility ($-\mu\text{m}\cdot\text{sec}^{-1}\cdot\text{V}^{-1}\cdot\text{cm}$) $\pm$ standard deviation for erythrocytes with time of incubation in $5\ \mu\text{g}/\text{ml}$ thiopental. At different time of incubation, an aliquot of cells were washed and then incubated in cultured medium containing serum (dashed lines).

Fig. 2. Change in the mean electrophoretic mobility ($\sim \mu\text{m} \cdot \text{sec}^{-1} \cdot \text{V}^{-1} \cdot \text{cm}$) $\pm$ standard deviation of cells with time of incubation in $1 \mu\text{g/ml}$ droperidol. At different time of treatment, an aliquot of cells were washed and then incubated in cultured medium containing serum (dashed line).

Fig. 3. Relationship between the concentration of anesthetics (Thiopental ---x---, Droperidol —○—) and the cell electrophoretic mobility after the treatment for 30 min. Vertical lines represent standard deviations of the mean.

Fig. 2 indicates the time-course alteration of EPM of erythrocytes incubated with $1 \mu\text{g/ml}$ droperidol (clinical concentration). Recovery in EPM after washing out of the drug was slower than with thiopental, but was almost complete in 24 hr.

Fig. 3 shows the relationship between the concentration of thiopental or droperidol and EPM of erythrocytes after the incubation for 30 min. The EPM reducing effect changed gradually with the increasing concentrations of thiopental ranging from 0.5 to $100 \mu\text{g/ml}$. In droperidol, however, the effect changed markedly from 0.3 to $1 \mu\text{g/ml}$. Neither of the anesthetics showed any substantial effect on EPM at concentrations lower than $0.3 \mu\text{g/ml}$.

Fig. 4 shows the reduction and recovery in EPM with time of incubation with $100 \mu\text{g/ml}$ thiopental. The erythrocytes showed almost complete reversibility

Fig. 4. Time-course change in the mean electrophoretic mobility ($-\mu\text{m} \cdot \text{sec}^{-1} \cdot \text{V}^{-1} \cdot \text{cm}$) $\pm$ standard deviation with incubation time in 100 $\mu\text{g/ml}$ thiopental. At different time of incubation, an aliquot of erythrocytes were washed and then incubated in cultured medium containing serum (dashed lines).

Fig. 5. Change in the mean cell electrophoretic mobility ($-\mu\text{m} \cdot \text{sec}^{-1} \cdot \text{V}^{-1} \cdot \text{cm}$) $\pm$ standard deviation with time of incubation in 10 $\mu\text{g/ml}$ droperidol.

in EPM up to 2 hr treatment with the drug. After 3 hr treatment, however, the recovery during the initial 2 hr was temporary, and EPM re-decreased in the next 2 hr. Treatment of the cells for 4 hr resulted in complete loss of reversibility.

Fig. 5 shows the time-course change with 10 $\mu\text{g/ml}$ droperidol. The recovery in EPM after washing out of the drug was evident up to 2 hr of treatment, but was weak or lacking after the treatment longer than 3 hr.

Fig. 6 illustrates the frequency distribution of EPM. Non-treated control cells have EPM ranging from 1.0 to 1.3. After 1 hr treatment with 100 $\mu\text{g/ml}$ thiopental, the distribution shifted down to EPM ranging from 0.6 to 1.0. The subsequent incubation for 24 hr after washing resulted in re-shift to almost normal distribution. Unrecovered fraction increased with increasing time of treatment from 2 to 4 hr.

Fig. 6. Frequency distribution of electrophoretic mobility of erythrocytes. Cells were treated different time with thiopental (100 $\mu\text{g}/\text{ml}$), washed, and then incubated 24 hr in culture medium RPMI+15% fetal calf serum: open columns (untreated control), diagonally striped columns (upper-1 hr treatment, lower-4 hr treatment-wash-24 hr incubation), shaded bars (upper-1 hr treatment-wash 24-hr incubation, lower-3 hr treatment-wash-24 hr incubation), stippled columns (2 hr treatment-wash-24 hr incubation).

With 10 $\mu\text{g}/\text{ml}$ droperidol, unrecovered fraction in 24 hr was greater than with thiopental.

DISCUSSION

Rapid decreases in electrophoretic mobility of rat erythrocytes by the incubation with thiopental or droperidol were observed at the clinical concentrations. These anesthetics and other drugs (xylocaine and procaine) showed the same effect on erythrocytes and all the cultured mammalian cells tested (unpublished data). The time course of mobility reduction and its recovery after washing out of the drug were dependent on the concentration and the kind of anesthetics. Droperidol, a long acting anesthetics, resulted in a slower recovery in electrophoretic mobility than short acting thiopental. At clinical concentrations, the recovery was almost complete in 24 hr post-incubation without drugs. At 10- to 20-fold high concentrations, however, 2 hr was the critical incubation time for retaining the reversibility.

Regarding the mechanism of mobility change, we are obtaining some evidence which suggests the conformation change of membrane by anesthetics; the amount of negatively charged sugars was not decreased, but its localization changed to a deeper layer. An involvement of contractile proteins of membrane (sensitive to colchicine, cytochalasin B or vinblastine) and calcium in the EPM reduction is also suggested (in preparation).

In general, the binding of anesthetics to the membrane is reversible (Seeman and Weinstein 1966). At high concentrations of anesthetics, however, irreversible exposure of new binding sites in the membrane and sharp rise of the membrane/buffer partition coefficient were reported (Colley et al. 1971; Metcalfe et al. 1968). The application of cell electrophoresis in this experiment provided new evidence for kinetic analysis of the relationship between the concentration of anesthetics and the membrane alteration. It clarified the term of 2 hr for retaining the reversibility.

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