Influence of the Light Schedule on the Toxic Interaction between Propranolol and Disopyramide in Chick Embryos

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The effect of the light schedule on toxic interactions between propranolol and disopyramide were studied in chick embryos. Fertilized eggs of White Leghorns were incubated under dark conditions and investigated, on two occasions, under light conditions or under dark conditions. Propranolol, with and without disopyramide, was injected into the air sac of fertilized eggs on the 16th day of incubation. Electrocardiograms (ECGs) were recorded 0 to 60 min after the injection. After the injection of propranolol with disopyramide, the heart rate was significantly decreased compared with the injection of propranolol alone under light conditions. In addition, this toxic interaction between propranolol and disopyramide was more severe under dark conditions than under light conditions. These findings indicate that manipulation of the light schedule has a marked influence on the toxic interaction between propranolol and disopyramide in chick embryos.

Key words light schedule; toxic interaction; chick embryo; propranolol; disopyramide; electrocardiogram

With the recent concern for animal rights, experimental studies using mammals have been limited in number and methods. Thus, based on social acceptance, experimental studies using chick embryos have drawn attention. Chick embryos have been widely used in pharmacological and toxicological experiments for evaluating drug action on the fetus.1–3)

In order to develop alternative methods, we have studied the biological effects of drugs on the cardiovascular system of chick embryos using physiological techniques.3–6)

Recently, many antiarrhythmic drugs have been put on the market in Japan and classified into subcategories. Such classification of antiarrhythmic mechanisms should prove of value in predicting the therapeutic as well as toxic effects of different agents, and may be particularly important in planning combination therapy for resistant arrhythmias. Drug–drug interactions have been demonstrated for a variety of drugs, including propranolol and disopyramide, in the treatment of heart failure patients.7,8)

We have evaluated the toxic interactions between propranolol and disopyramide in chick embryos.9)

Circadian-dependent changes in pharmacological effects have been demonstrated for a variety of drugs, including some antiarrhythmic drugs.10) The light schedule is undoubtedly an important determinant in demonstrating the circadian rhythm of drug actions.

The present study evaluated the effect of the light schedule on the toxic interaction between propranolol and disopyramide in chick embryos.

MATERIALS AND METHODS

Fertilized eggs of White Leghorns (Omiya Poultry Laboratory, Saitama, Japan) were incubated at 37.5 ± 0.2 °C at a relative humidity of about 65% under dark conditions. An experiment was performed under light conditions (under the fluorescent lamp, 450 lux) or dark conditions (under a red safety lamp, 12 lux) for 2 h after acclimatization.

Propranolol preparation (Sumitomo Pharmaceutical, Tokyo, Japan) and disopyramide preparation (Chugai Pharmaceutical, Tokyo, Japan) were used for the treatment. Propranolol at 0.1 mg/egg and disopyramide at 0.3 or 1.0 mg/egg were injected into the air sac of each fertilized egg on the 16th day of incubation. After the injection of propranolol with and without disopyramide under light conditions or dark conditions, the heart rate values were measured.

Electrocardiograms (ECGs) were recorded 0 to 60 min after the drug injection, and heart rate was determined from RR intervals. Changes in heart rate were expressed as mean percent changes of the drug-treated groups compared to the matched control.

Four small holes every 90 degrees on “the equator” and one small hole on “the south pole” were made on each fertilized egg by an electric drill, then sealed with paraffin (mp 60 °C). Specially designed electro-needles were inserted into the appropriate holes of “the equator” and “the south pole.” The two needles on “the equator” were used as a bipolar lead of the embryonic heart, and the needle on “the south pole” was used as a ground lead. These needles were connected to the electrocardiograph system (Nihon Koden AVB-21, Tokyo, Japan). ECGs were recorded as bipolar waves between the two needles on a thermal array recorder (Nihon Koden PTA-1100M, Tokyo, Japan) with a paper speed of 25 mm/s.

The data were analyzed by one way analysis of variance. If there was a significant difference among the groups, a multiple comparison test was conducted (Tukey’s test). The fiducial limit of 0.05, two-tails, was used as the criterion to determine significance.

RESULTS

In the non-drug state, the heart rate of the chick embryos was not significantly different between the light (229.7 ± 10.0) and dark (211.3 ± 9.0) conditions. Moreover, there were no significant differences in the growth of the chick embryos at each light schedule, and the body weights of chick embryos gradually increased with the day of incubation.
Fig. 1. Changes in Heart Rate of Chick Embryo after Administration of Propranolol with and without Disopyramide under Light Conditions or Dark Conditions

Propranolol 0.1 mg/egg alone (light; ○, dark; □), propranolol 0.1 mg/egg plus disopyramide 0.3 mg/egg (light; △, dark; ▲) or propranolol 0.1 mg/egg plus disopyramide 1.0 mg/egg (light; ○, dark; ◼) was injected into the air sac of fertile eggs on the 16th day of incubation. Changes in heart rate were presented as mean percent changes of drug-treated groups over the time-matched control. Each point represents the mean and standard error for six rats. # Significantly different from propranolol 0.1 mg/egg plus disopyramide 0.3 mg/egg under light condition group, p<0.05. * Significantly different from propranolol 0.1 mg/egg plus disopyramide 1.0 mg/egg under light condition group, p<0.05. The heart rates of chick embryos before each drug injection: propranolol 0.1 mg/egg alone under light condition (220±15 beats/min) and under dark condition (214±15 beats/min), propranolol 0.1 mg/egg plus disopyramide 0.3 mg/egg under light condition (227±4 beats/min) and under dark condition (220±20 beats/min), propranolol 0.1 mg/egg plus disopyramide 1.0 mg/egg under light condition (224±10 beats/min) and under dark condition (223±18 beats/min).

After the injection of propranolol with disopyramide, the heart rate was significantly decreased compared with those injected with propranolol alone under light conditions. In addition, this toxic interaction between propranolol and disopyramide was more severe under dark conditions than under light conditions (Fig. 1).

DISCUSSION

We have demonstrated in this report that our recording system for an electron cardiogram system using chick embryos may be applied as an animal test alternative. The chick embryonic heart develops through a similar process as in mice, rats and humans, and also has a similar atrioventricular system for an electron cardiogram system using chick embryos.

In conclusion, our in vivo recording system for ECG of chick embryos may be useful for investigating the toxic interactions of cardiovascular drugs. In addition, the manipulation of the light schedule and the timing of drug-drug interactions in relation to these lighting conditions may help us to design rational chronopharmacology of some drug-drug interactions, including some antiarrhythmic drugs, under certain experimental situations.

REFERENCES

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