Influence of Hypothyroidism Induced by Thiamazole on the Toxicity of Amitriptyline in Chick Embryos

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The effect of hypothyroidism induced by thiamazole on the toxicity of amitriptyline was studied in chick embryos. Fertilized eggs of White Leghorns were incubated and investigated. 1.2 mg/0.2 ml/egg of thiamazole was injected into the albumen of fertilized eggs on the 9th day of incubation. The control group was given 0.2 ml/egg of physiological saline in the same manner. Amitriptyline at 1 mg/egg was injected into the air sac of fertilized eggs on the 16th day of incubation. Electrocardiograms were recorded 0 to 60 min after the injection. After the injection of amitriptyline into the thiamazole-treated eggs, the heart rate was significantly decreased compared with the untreated eggs. These findings indicate that hypothyroidism induced by thiamazole has a marked influence on the toxicity of amitriptyline in chick embryos.

Key words hypothyroidism; thiamazole; cardiotoxicity; chick embryo; amitriptyline; electrocardiogram

With regard to the use of experimental animals for research and education, alternative methods for animal testing came to be discussed on account of criticisms made on animal welfare and humanitarian grounds as well as awareness among scientists.[1] Thus, based on social acceptance, experimental studies using chick embryos have drawn attention. In order to develop alternative methods, we have studied the biological effects of drugs on the cardiovascular system of chick embryos using physiological techniques.[2—6]

An experimental animal model with heart disease originating from abnormalities of the thyroid gland in chick embryos has been produced by treatment with thiamazole and the pharmacological and toxicological effects of cardiotonics were examined using this model.[7,8] The pharmacological and toxicological activities of thiamazole have characteristics in common with that of thiourea. It has been reported that when thiourea derivatives were injected into the albumen of eggs, the time of the injection strongly affected in the body weight and thyroid gland weight from the 9th to 12th day of incubation.[9] In addition, they showed that that a state of hypothyroidism could be produced in chick embryos by injection of these drugs.

Amitriptyline is known to alter myocardial function manifested by electrocardiogram changes. Although tricyclic antidepressants may have cardiovascular effects at the therapeutic doses, it is generally thought to be dose-related and widening of the QRS complex is reported to correlate well with the severity of toxicity following acute overdose ingestion.[10,11] We have evaluated the toxic interactions between propranolol and disopyramide in chick embryos.[12,13]

The present study evaluated the effect of hypothyroidism induced by thiamazole on the toxicity of amitriptyline 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%, turned automatically every hour.

Thiamazole (Chugai Pharmaceutical, Tokyo, Japan) and amitriptyline hydrochloride (Banyu Pharmaceutical Co, Ltd., Tokyo) were used for the treatment. 1.2 mg/0.2 ml/egg of thiamazole was injected into the albumen of fertilized eggs on the 9th day of incubation. The control group was given 0.2 ml/egg of physiological saline in the same manner.

Amitriptyline at 1 mg/egg, 2.5 mg/egg or 5 mg/egg was injected into the air sac of the thiamazole untreated eggs on the 16th day of incubation (six eggs in each group). Amitriptyline at 1 mg/egg was injected into the air sac of the thiamazole-treated eggs or the untreated eggs on the 16th day of incubation (six eggs in each group).

After the injection of amitriptyline into the eggs, the heart rate values were measured.

Electrocardiograms (ECGs) were recorded 0 to 60 min after drug injection, and heart rate was determined based on R-R intervals. Changes in heart rate were expressed as mean percentage changes in the drug-treated groups compared with the matched control. Four small holes were made at 90-degree intervals in "the equator," as well as one small hole in "the south pole," and one small hole in "the north pole" of each fertilized egg using an electric drill, and then they were all sealed with paraffin (mp 60 °C). Specially designed needle electrodes were inserted into the appropriate holes of the equator and the south pole. Two needles on the equator were used as a bipolar lead from the embryonic heart, and the needle on the south pole was used as a ground lead. These needles were connected to a memory oscilloscope (VC-11, Nihon Koden Co., Tokyo). ECGs were recorded as bipolar waves between two needles on a recorder (PowerLab System, ADInstruments Japan Co., Tokyo) (Fig. 1).

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

The body weight of chick embryos gradually increased with the day of incubation. After the administration of amitriptyline 1 mg/egg, the heart rate was not different com-

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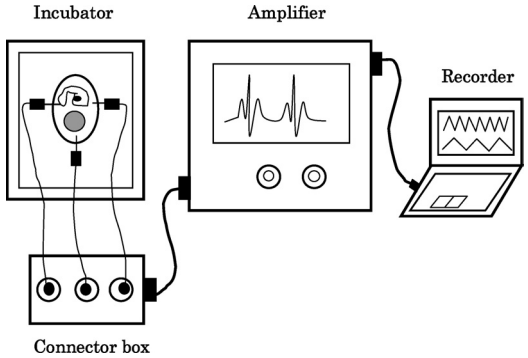

Fig. 1. Schema of ECG-Recording System for Chick Embryo in Egg Shell

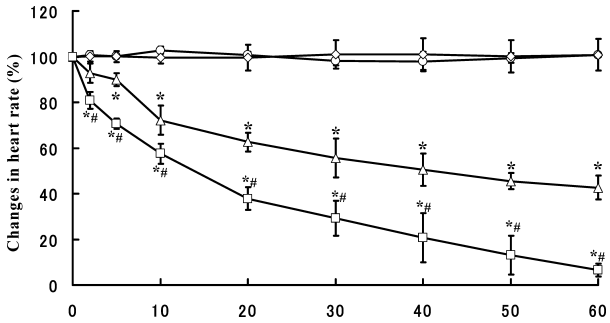

Fig. 2. Heart Rate of Chick Embryo after Administration of Amitriptyline Alone in Untreated Eggs

Saline (◇), amitriptyline 1 mg/egg (○), 2.5 mg/egg (△) or 5 mg/egg (□) was injected into the air sac of fertile eggs on the 16th day of incubation. Changes in heart rate are presented as mean percent changes of drug-treated groups over the time-matched control. Each point represents the mean and S.D. (bar) for six eggs. * Significantly different from saline group, $p<0.05$. # Significantly different from amitriptyline 1 mg/egg group, $p<0.05$.

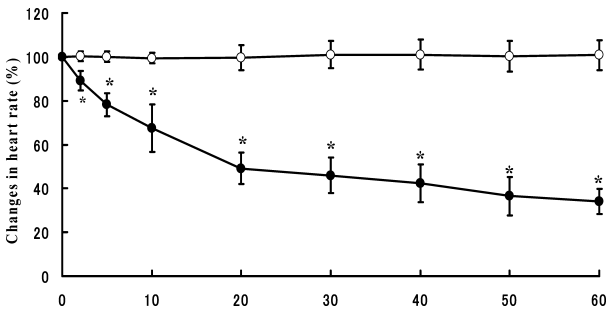

Fig. 3. Heart Rate after Administration of Amitriptyline in Chick Embryos with Hypothyroidism Induced by Thiamazole

Amitriptyline 1 mg/egg in untreated eggs (○) or thiamazole-treated eggs (●) was injected into the air sac of fertile eggs on the 16th day of incubation. Heart rates are presented as the mean percent changes of drug-treated groups over the time-matched control. Each point represents the mean and S.D. (bar) of 6 eggs. * Significantly different from the untreated eggs, $p<0.05$.

pared with physiological saline. However, the heart rate was significantly decreased by the administration of 2.5 mg/egg and 5 mg/egg amitriptyline (Fig. 2). After the injection of amitriptyline into the thiamazole-treated eggs, the heart rate was significantly decreased compared with the untreated eggs (Fig. 3).

DISCUSSION

Amitriptyline is a tricyclic antidepressant and may be used in the treatment of depression. The mechanism of action of amitriptyline is thought to inhibit the membrane pump mechanism responsible for uptake of norepinephrine and serotonin in adrenergic and serotonergic neurons.[14] Newer classes of antidepressants are not more effective than amitriptyline. It has been demonstrated that imipramine possesses quinidine-like properties, probably by a direct effect on myocardial cells.[15] Caution should be exercised when prescribing antidepressants to the elderly as they seem to be less tolerant to the cardiac effects.

Cardiotoxicity of amitriptyline was demonstrated in chick embryos. Amitriptyline led to a QTc interval prolongation of the ECGs. After the drug was injected into the air sac of each fertilized egg, it accumulated in the eggshell. Therefore, the heart rate may be decreased in a time-dependent manner.

The toxicological and pharmacological effects of cardiovascular drugs are usually studied in mammals and the results obtained are extrapolated to humans. Chick embryonic heart develops through a similar process to that in mice, rats and humans, and also has a similar atrioventricular system.[16] Chick embryos have been widely used in pharmacologic and toxicologic experiments for evaluating drug actions on the fetus.[17]

We have also reported that the chick embryonic model of hypothyroidism produced by treatment with thiamazole can be used to examine the pharmacological and toxicological effects of cardiovascular drugs.[7,8]

In the present study, the effects of hypothyroidism induced by thiamazole on the toxicity of amitriptyline were investigated in chick embryos, and it was found the hypothyroidism induced by thiamazole modified the toxicity of amitriptyline in the chick embryos.

The antithyroid drugs thiamazole and propylthiouracil have been widely used as therapeutic drugs in patients with hyperthyroidism. Toxicological studies have shown that antithyroid drugs at overdose levels cause functional and morphological changes in the thyroid in rats and dogs.[18,19] In addition, it has been reported that functional abnormalities of the thyroid gland are often accompanied by heart disease and can show unexpected responses to cardiotonics, such as digoxin.[20] Therefore, to predict the effects of cardiovascular drugs, experimental animals with a very sensitive heart condition such as hypothyroidism or hyperthyroidism should be used. A convenient thyrotoxic model would be of great benefit for evaluating the side effects and toxicity of cardiovascular drugs. As thyroid hormone has multiple functions in chick embryos as well as in mammals, further investigation is necessary to clarify the mechanism of cardiotoxicity in chick embryonic hypothyroidism.

In conclusion, our *in ovo* ECG recording system in chick embryos may be useful for investigating the toxicity of amitriptyline. In addition, thiamazole-treated chick embryos may prove to be an alternative animal model under certain experimental situations with which the cardiotoxicity of some drugs, including amitriptyline, might be examined.

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REFERENCES

1) Ohno Y., *Altern. Animal Test. Experiment.*, **11**, 1—3 (2005).
2) Yoshiyama Y., Sugiyama T., Kanke M., *Biol. Pharm. Bull.*, **26**, 893—895 (2003).
3) Yoshiyama Y., Sugiyama T., Kanke M., *Altern. Animal Test. Experiment.*, **9**, 40—44 (2003).
4) Yoshiyama Y., Sugiyama T., Tsuchimoto K., Kanke M., *Biol. Pharm. Bull.*, **27**, 128—130 (2004).
5) Yoshiyama Y., Kanke M., *Biol. Pharm. Bull.*, **28**, 151—153 (2005).
6) Yoshiyama Y., Sugiyama T., Kanke M., *Biol. Pharm. Bull.*, **28**, 1986—1988 (2005).
7) Sugiyama T., Saito K., Shimada H., Tsuchimoto K., Yoshiyama Y., *Altern. Animal Test. Experiment.*, **6**, 89—96 (2000).
8) Yoshiyama Y., Kanke M., *Biol. Pharm. Bull.*, **28**, 1983—1985 (2005).
9) Romanoff A. L., Romanoff A. J., "Antithyroid Drugs, in Pathogenesis of the Avian Embryo," Wiley-Interscience, John Wiley & Sons, Inc., New York, 1972, pp. 305—310.
10) Robinson D. S., Barker E., *JAMA*, **236**, 2089 (1976).
11) Biggs J. T., Spiker D. G., Petit J. M., *JAMA*, **238**, 135—138 (1977).
12) Yoshiyama Y., Sugiyama T., Kanke M., *Biol. Pharm. Bull.*, **27**, 223—225 (2004).
13) Yoshiyama Y., Sugiyama T., Kanke M., *Altern. Animal Test. Experiment.*, **10**, 18—23 (2004).
14) Product Information: Elavil(R), amitriptyline. Merck & Co, Inc, West Point, PA, (PI revised 12/1999) reviewed 1/2003 (2003).
15) Bigger J. T., Giardino C. G., Perel J. M., *N. Engl. J. Med.*, **296**, 206—208 (1977).
16) Bulter H., Juurlink B. H. J., "An Atlas for Staging Mammalian and Chick Embryos," CRC Press, Inc., Boca Raton, 1987, pp. 19—138.
17) Rajala G. M., Kuhlmann R. S., Kolesari G. L., *Teratology*, **30**, 385—392 (1984).
18) Dowell R. T., Atkins F. L., Love S., *Methods Find. Exper. Clin. Pharmacol.*, **14**, 507—515 (1992).
19) Shigemasa C., Mitani Y., Taniguchi S., Adachi T., Ueta Y., Urabe K., Miyazaki S., Tanaka T., Yoshida A., Mashiba H., *Arch. Intern. Med.*, **150**, 1105—1109 (1990).
20) Marrow D. H., Gafeney T. E., Graunwall D. E., *J. Pharmacol. Exp. Ther.*, **140**, 324—328 (1963).