Altern. Animal Test. Experiment. 6 (1),72-78 (1999)

Utilization of Chick Embryonic Electrocardiograms to Detect the Pro-arrhythmic Actions by Antiarrhythmic Drugs

Takashi Sugiyama, Hiroyuki Miyazaki and Hideyo Shimada

Division of Pathophysiology, Center for Clinical Pharmacy and Clinical Sciences, School of Pharmaceutical Sciences, Kitasato University, 9-1, Shirokane 5-chome, Minato-ku, Tokyo 108-8641, Japan

Abstract

We have studied the pharmacological and toxicological effects of cardiovascular drugs on chick embryos using electrocardiograms in order to develop alternatives to the use of mammals. Recently, proarrhythmic actions induced by antiarrhythmic drugs have been widely studied. In this study, we investigated the pharmacological effects of antiarrhythmic drugs, Ia (procainamide), Ib(lidocaine), Ic(flecainide), II(propranolol) and IV(verapamil) types, as classified by Vaughan Williams. When a single injection of drugs was performed into the air sac of fertile eggs of White Leghorns on the 16th day of incubation, all drugs decreased HR in a dose-dependent manner. Also, when higher dosages of drugs were injected, these drugs induced various kinds of arrhythmias, i.e., S-A or A-V blocks, as shown in mammals. The intervals of PQ, QRS and QT waves varied depending on the drugs used. However, the ECG waves exhibited the same changes seen in mammals with a few exceptions. Our ECG recording system using chick embryos may be applicable for screening tests for proarrhythmic actions of antiarrhythmic drugs.

Keywords: *Chick embryo, alternative animal, antiarrhythmic drug, electrocardiogram, proarrhythmic action*

Introduction

We have evaluated the pharmacological and toxicological effects of cardiovascular drugs on chick embryos using physiological techniques including electrocardiograms and echocardiograms in order to develop alternative methods to the use of experimental animals(Sugiyama et al., 1996, Miyazaki. et al., 1994, Yoshiyama et al., 1997, Saito et al., 1993)

Previously, we reported that tachycardia in chick embryos induced by isoprenaline is completely inhibited by the β-adrenergic

Correspondence: Takashi Sugiyama, Ph.D.
Division of Pathophysiology, Center for Clinical Pharmacy and Clinical Sciences, School of Pharmaceutical Sciences, Kitasato University, 9-1, Shirokane 5-chome, Minato-ku, Tokyo 108-8641, Japan
Tel and Fax; +81-03-3446-9036
E-mail; sugiyamat@platinum.pharm.kitasato-u.ac.jp

receptor blocking agent propranolol (Sugiyama et al., 1997). At that time, we did not examine proarrhythmic actions induced by antiarrhythmic drugs. Recently, since the reports of The Cardiac Arrhythmia Suppresion Trial (CAST), proarrhythmic actions induced by antiarrhythmic drugs have been widely studied (CAST Investigators. 1989, CAST II Investigators. 1992).

The pharmacological effects of antiarrhythmic drugs show a different attitude among human, dog and rat. However, it was unknown whether proarrhythmic actions could be induced by injection of antiarrhythmic drugs into chick embryos or whether the arrhythmias of chick embryos are similar to those in mammals.

In this study, we analyzed the ECG waves in chick embryos induced by type I antiarrhythmic drugs, Ia (procainamide), Ib (lidocaine), Ic(flecainide), type II(propranolol) and type IV(verapamil) as classified by Vaughan Williams, and we attempted to predict the proarrhythmic actions by comparing changes between the ECG in the chick embryos and those in rats and in man.

Materials and Methods

Eggs and incubation

White Leghorn fertile eggs were incubated at 37.5°C at a relative humidity of about 65.5% and were turned automatically every hour (P-1 type, Showa Incubator Laboratory). Eggs were used on the 16th day of incubation for this experiment.

Antiarrhythmic drugs used

Procainamide hydrochloride (Daiichi Pharmaceutical Co., Ltd., Tokyo), lidocaine hydrochloride (Fujisawa Pharmaceutical Co., Ltd., Osaka), flecainide acetate(Eisai Co., Ltd., Tokyo), propranolol(Sumitomo Pharmaceutical Co., Ltd., Osaka), and verapamil hydrochloride (Eisai Co.,Ltd., Tokyo) were purchased from commercial sources and were diluted in sterilized physiological saline to the desired concentrations in 0.2 mL of solution.

Injection of drugs and ECG recording in chick embryos

Anesthetic injection of urethane ((45 mg / egg) + α-chloralose (4.5mg/egg)) was performed into the air sac of eggs to record the stable ECG waves in 16-day-old embryos which could be clearly analyzed RR, PQ, QRS and QT intervals. Drugs were injected into the air sac of eggs in a volume of 0.2 mL 20 min after injection of the anesthetic. As described in the previous paper (Sugiyama et al., 1996), specially designed needle electrodes were inserted into two small holes at the equator and one at South Pole. Two needles on the equator were used as the bipolar lead for the embryonic heart and one needle at the South Pole was used as a ground lead. Three needles were connected to an ECG recording system (Nihon Koden AVB-21, Tokyo, Japan) and the ECG waves were recorded up to 60 min for 15 sec every 2-10 min. The recorder paper speed was 25 mm/s or 100 mm/s. The intervals of RR, PQ, QRS and QT waves were measured using calipers.

Statistical analysis: Values are expressed as mean ± S.E.M. Student's paired t-test was used for statistical evaluation, and P values smaller than 0.05 were considered to be significant.

Results

Incidence of arrhythmia in the chick embryos induced by drugs

All five antiarrhythmic drugs used in this experiment induced arrhythmias in the chick embryos in dose- and time-dependent manners (Table 1).

In the embryos treated with verapamil (0.025 and 0.075mg/egg), arrhythmia was seen in some embryos 2 min after injection and was seen in all embryos (six out of six embryos) at 60 min. The frequency of arrhythmia in the

Table 1. Frequency of arrhythmia in chick embryo treated with antiarrhythmic drugs

Drugs	Class	Dose	Time(min) after injection					
		(mg/egg)	2	5	10	20	40	60
		3	0	0	0	0	0	0
Procainamide	Ia	10	0	0	0	0	0	0
		30	0	0	2	2	2	4
		0.15	0	0	0	0	0	0
Lidocaine	Ib	0.5	0	0	0	0	0	3
		1.5	0	0	1	2	3	3
		0.3	0	0	0	0	0	0
Flecainide	Ic	1	0	0	1	2	2	3
		3	0	1	4	4	4	4
		0.03	0	1	0	0	0	2
Propranolol	II	0.1	0	0	0	0	1	2
		0.3	0	0	0	0	2	2
		0.0075	0	0	0	0	0	0
Verapamil	IV	0.025	1	1	1	1	2	2
		0.075	1	2	2	3	6	6

Drugs were injected into the air sac of eggs(n=6) on the 16th day of incubation and ECGs were recorded until 60 min after injection.

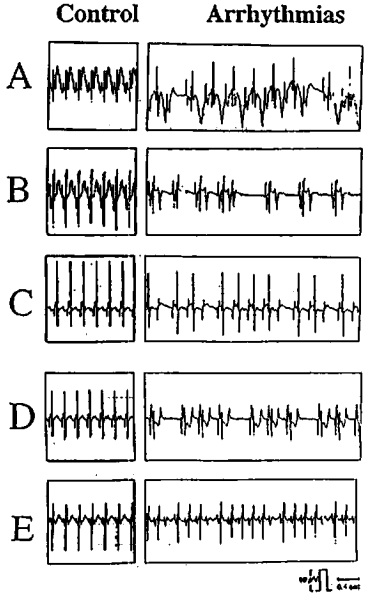

Fig. 1. Arrhythmias in ECG waves in chick embryos treated with antiarrhythmic drugs

Drugs were injected into the air sac of fertile eggs on the 16th day of incubation and ECG waves were recorded until 60 min after injection. A:procainamide; II degree A-V block (Mobitz type, at 40 min after injection of 30 mg/egg). B:lidocaine; sinus arrhythmia (30 min, 1.5 mg). C: flecainide; II degree A-V block (Mobitz, 20 min, 3 mg). D: propranolol; S-A block (60 min, 0.1 mg). E: verapamil; II degree A-V block (Mobitz, 10 min, 0.025 mg).

chick embryos at the highest dose level was 66.6% by procainamide, 50% by lidocaine, 66.6% by flecainide and 33.3 % by propranolol. Various types of arrhythmia in the chick embryos treated with antiarrhythmic drugs are shown in Fig.1.

Second degree A-V block was seen in 100% of cases in arrhythmias treated with 30 mg/egg of procainamide or 0.075 mg of verapamil and 50% of cases (3/6) in arrhythmias treated with 3mg of flecainide. These A-V blocks were mainly Mobitz type. However, a case of Wenckebach type was observed after treatment with 3 mg flecainide (data not shown). S-A block was observed in 33.3% of the cases treated with propranolol, regardless of dose levels. Sinus arrhythmia (2 out of 6) and aberrant ventricular conduction (1/6) were observed in cases treated with 1.5 mg of lidocaine.

Changes in heart rate and the parameters of ECG waves in chick embryos treated with antiarrhythmic drugs

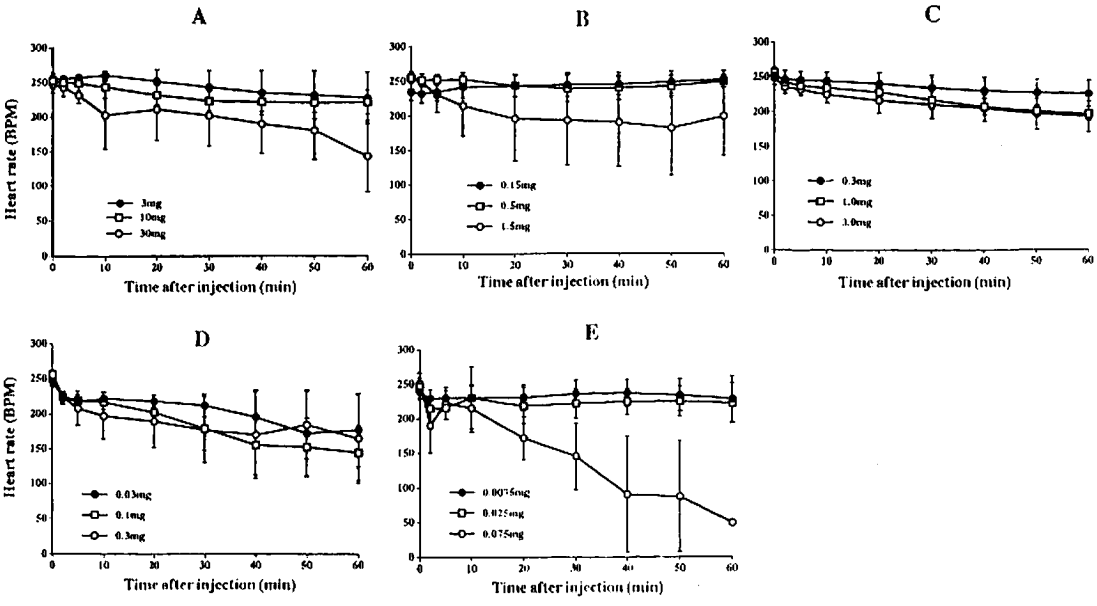

Fig. 2. Changes in heart rate in chick embryos treated with antiarrhythmic drugs

Drugs were injected into the air sac of fertile eggs on the 16th day of incubation. Values are the mean ± S.E.M. *; $p<0.05$, significantly different from 0 time on each point by a paired Student's t-test.

As shown in Fig.2, all five antiarrhythmic drugs decreased HR in a dose-dependent manner.

The PQ, QRS and QT intervals in the ECG waves of the chick embryos subjected to the highest and lowest doses of each drug except verapamil were measured. Time-response curves of RR, PQ, QRS and QT intervals after injection of antiarrhythmic drugs are shown in Fig.3.

Procainamide (30 mg/egg) and flecainide (3 mg/egg) caused a significant prolongation in PQ, QRS and QT intervals during arrhythmia. In lidocaine treatment cases, a significant prolongation of QRS and QT intervals were shown at various times after injection. Propranolol decreased the HR with characteristic prolongation in QT intervals, but did not change the QRS interval. Although injection of the intermediate dose of verapamil did not decrease the HR and hardly changed the QRS and QT intervals, a prolongation of PQ intervals was observed at various times after injection.

Discussion

Our studies showed that chick embryos treated with antiarrhythmic drugs exhibit proarrhythmic effects as shown by ECG waves. ECG analysis also revealed significant differences in arrhythmias induced by these drugs. This study was initially designed to identify changes in heart rate which decrease in a dose-response manner after treatment. However, when the ECG waves in the treated chick embryos were analyzed, various ECG wave cycles, including arrhythmias, PQ, QRS and QT intervals were observed, depending on the drug used. Further, these drugs induced proarrhythmic effects including bradycardia with AV or SA blocks at middle and/or high dose levels. We also compared chick embryonic data with those in man (Beaven and Thompson. 1983a, Anderson et al., 1984, Kjekshus et al., 1984), and in rat (Suzuki et al., 1991) to analyze whether chick embryos can be used to predict the proarrhythmic actions of antiarrhythmic drugs in man (Table 2).

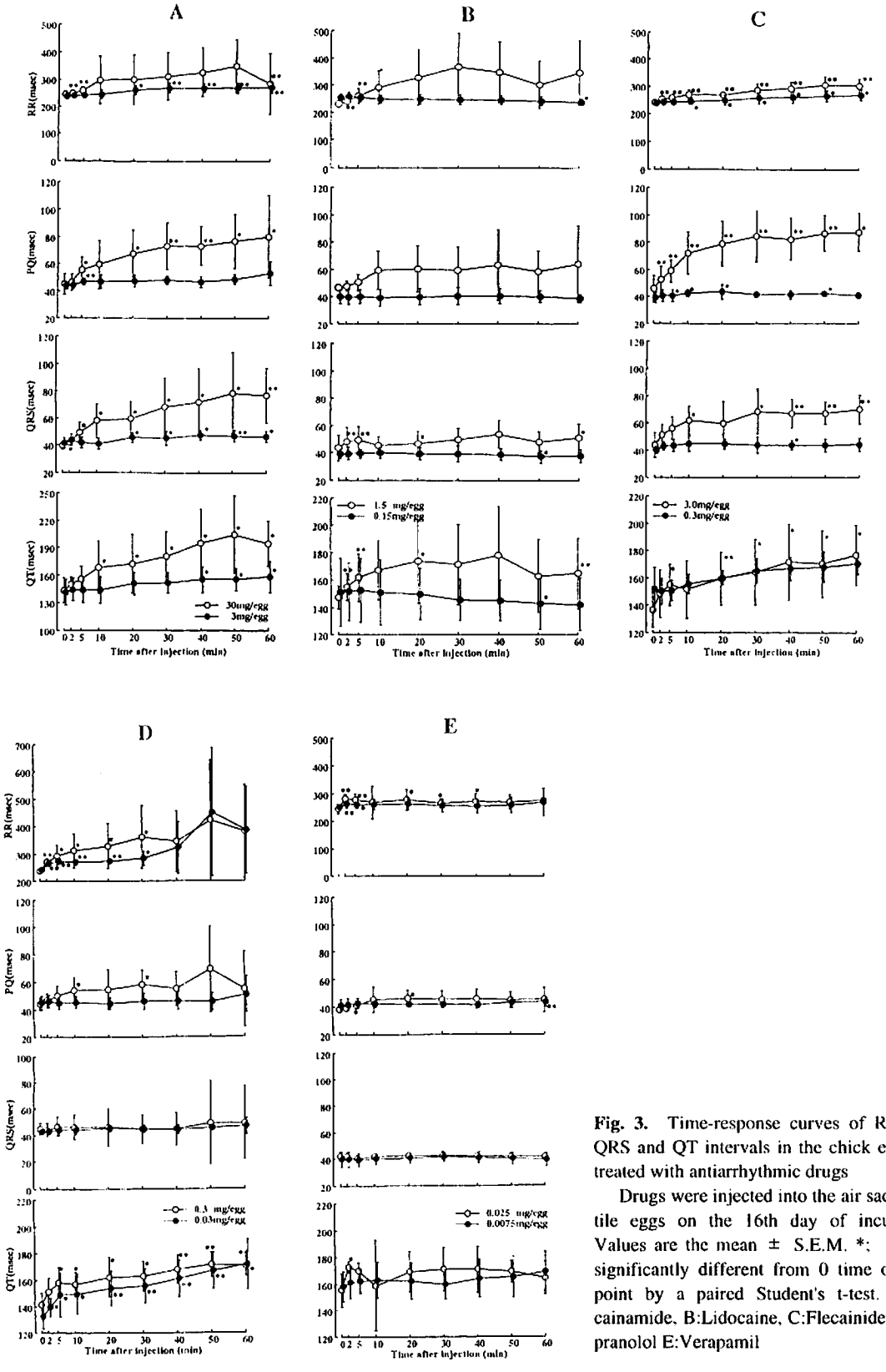

Fig. 3. Time-response curves of RR, PQ, QRS and QT intervals in the chick embryos treated with antiarrhythmic drugs

Drugs were injected into the air sac of fertile eggs on the 16th day of incubation. Values are the mean ± S.E.M. *; $p<0.05$, significantly different from 0 time on each point by a paired Student's t-test. A:Procainamide, B:Lidocaine, C:Flecainide D:Propranolol E:Verapamil

Table 2. Changes in ECG parameters in chick embryo, rat and man treated with antiarrhythmic drugs

Drugs	Class	Qualitative changes in ECG parameters			
		RR	PQ	QRS	QT
Procainamide	I a	⇑⇑(↑,⇧)	⇑⇑(↑,⇧)	⇑⇑(↑,⇧)	⇑⇑(↑,⇧)
Lidocaine	I b	⇑⇑(↑,⇨/⇧)	⇑(→,⇨)	⇑⇑(↑,⇧)	⇑⇑(↑,⇩)
Flecainide	I c	⇑⇑(⇧)	⇑⇑(⇧)	⇑⇑(⇧)	⇑⇑(⇧)
Propranolol	II	⇑⇑(↑,⇧)	⇑(↑,⇨/⇧)	➡(↑,⇧)	⇑(↑,⇩)
Verapamil	IV	⇑(↓,⇧)	⇑(↑,⇧)	➡(→,⇨)	➡(↑,⇨)

↑ ↑ :significant prolongation ↑ :prolongation, ↓ :shortening, →:no change
➡ :chick embryo
(→):rat
(↳):human

There are currently 3 I type antiarrhythmic drugs which have been classified by the different speeds at which they reduce the transfer of sodium across the myocardiac cell membrane; Ia (procainamide) is the most rapid, Ic (flecainide) is the slowest and Ib (lidocaine) is between Ia and Ic. Procainamide decreased the HR in chick embryos in a dose-dependent manner and at the highest dose induced an arrhythmia (AV block) and prolongation of all ECG parameters. These results are quite similar to previous results in man and rat, as shown in Table 2. Lidocaine decreased the HR at the highest dose level and induced sinus arrhythmia associated with a prolongation of QRS and QT intervals. In man and rats, lidocaine only rarely causes sinus arrest (Rosen and Wit, 1983), but arrhythmia with a prolongation of QT and QRS intervals has been reported.

Flecainide is an antiarrhythmic drug which was associated with an increased incidence of sudden cardiac death in patients with heart failure in the CAST study. When at least one mg/egg of flecainide was injected into the eggs, arrhythmias (A-V block) were induced as well as a significant prolongation of PQ, QRS and QT intervals. Katristic et al (1995) has reported that the prolongation of the QT and QRS intervals in patients with heart failure was observed after treatment with flecainide. It may be possible to predict the proarrhythmic actions in mammals induced by type I antiarrhythmic drugs using ECG waves in chick embryos. Further, these changes in the ECG parameters are probably related to the reflection of the conduction disturbances in the myocardium.

Propranolol is a type II antiarrhythmic drug which causes β-adrenergic receptor blockage. Propranolol treatment of chick embryonic heart caused S-A block and a prolongation of the PQ and QT intervals during a prolongation of RR intervals, but did not effect the QRS complex. Suzuki et al (1991) has reported that propranolol caused prolongation of QRS complex in rat. Small differences in ECG parameters between chick embryos and mammals may be due to differences in species.

Verapamil is a type IV antiarrhythmic drug which interferes with calcium channels. Injection of verapamil has been shown to frequently cause II degree A-V block in ECG waves which are classified as Mobitz type in the bradycardia. The same phenomenon has been observed in man (Bevan and Thompson, 1983b).

Our data is in good agreement with observations in man, including decresed HR, conduc-

tive disturbances in myocardial cells, and similar changes in ECG parameters in chick embryos treated with procainamide, flecainide and verapamil. While lidocaine and propranolol decreased the HR with a prolongation of QT intervals as in rat, a part of ECG parameters was not similer.

In conclusion, although the exact mechanism underlying the influence of antiarrhythmic drugs on the proarrhythmic actions in the chick embryonic heart remains to be clarified, our ECG recording systems using chick embryos may be applicable for screening proarrhythmic actions of antiarrhythmic drugs.

References

Anderson J. L., Stewart J. R.and Crevey B.J. (1984) A proposal for the clinical use of flecainide, *Am. J. Cardiol.*, 53, 112B-119B.

Bevan.J.A and Thompson.J.H (1983a) in *Essentials of Pharmacology*, 3 ed, pp.347, Happer & Row, Publishers Inc., Philadelphia.

Bevan.J.A and Thompson.J.H (1983b) in *Essentials of Pharmacology*, 3rd ed., pp.351, Happer & Row, Publishers Inc., Philadelphia.

Cardiac Arrhythmia Suppression Trial (CAST) Investigators (1989) Preliminary report: effect of encainide and flecainide on mortality in randomized trial arrhythmia suppression after myocardial infarction, *New. Engl.J. Med.*, 321, 406-412.

CAST II Investigators (1992) Effect of the antiarrhythmic agent moricizine on survival after myocardial infarction, *New.Engl.J. Med.*, 327, 227-233.

Katritsis, D., Rowland, E., O'Nunain, S., Shakespeare, C.F., Poloniecki, J. and Camm, A.J.(1995) Effects of flecainide on atrial and ventricular refractoriness and conduction in patients with normal left ventricle, *Eur. Heart J.*, 16, 1930-1935.

Kjekshus, J., Bathen, j., Orning, O.M., and Storstein, L.(1984) A double-blind crossover comparison of flecainide acetate and disopyramide phosphate in the treatment of ventricular premature complexes, *Am. J. Cardiol.*, 53, 72B-78B.

Miyazaki, H., Sugiyama, T., Saito, K., Kubota, N., Yoshiyama, Y. and Shimada, H.(1994) Toxicologic study of cardiovascular drugs in chick embryos and rodents, *In vitro Toxicol.*, 7, 243-246.

Rosen, M.R. and Wit, A.L.(1983) Electropharmacology of antiarrhythmic drugs, *Am. Heart J.*, 829-839.

Saito, K., Sugiyama, T., Kubota, N. and Shimada, H.(1993) Pharmacological and Toxicological studies using chick embryos (2) Estimation of drugs induced abnormalities in cardiovascular systems of chick embryos using color Doppler echocardiography, *Altern. Animal Test. Experiment.*, 2, 85.

Sugiyama, T., Miyazaki, H., Saito, K. and Shimada, H.(1997) The pharmacological effect of b-blockers can be evaluated by using a chick embryo tachycardia model, in *Animal Alternatives, Welfare and Ethics*, ed. by L.F.M.van Zutphen and M.Balls, pp.909-911, Elsevier Science B.V.

Sugiyama, T., Miyazaki, H., Saito, K., Shimada, H. and Miyamoto, K.(1996) Chick embryos as an alternative experimental animal for cardiovascular investigations: stable recording of electrocardiogram of chick embryos in ovo on the 16th day of incubation, *Toxicol. Appl. Pharmacol.*, 138, 262-267.

Suzuki, J., Tsubone, H. and Sugano, S.(1991) Characteristics of electrocardiographic changes with some representative antiarrhythmic drugs in adult rats, *J.Vet. Med. Sci.*, 53, 779-578.

Yoshiyama, Y., Sugiyama, T., Miyazaki, H., Shimada, H. and Ohdo, S.(1997) Influence of the lighting on the toxicity of doxorubicin in chick embryos, *Altern. Animal Test. Experiment.*, 4, 55-61.