PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 150, 240–243 (1975)

Effects of Diazepam on the Isolated Chick Embryo Heart[1] (39011)

DOUGLAS G. BERRY
(Introduced by F. E. Shideman)

Departments of Anesthesiology and Pharmacology, University of Minnesota Health Sciences Center, Minneapolis, Minnesota 55455

Diazepam has been widely used clinically for numerous conditions, among them being cardioversion (1–8), status epilepticus (9–11), and induction of anesthesia (12–14). Reports concerning the effects of this drug on cardiac function have been varied and often contradictory. Reports of actual stimulation of cardiac functions by diazepam are infrequent. Abel *et al.* (15) found that diazepam augmented myocardial contractility in anesthetized dogs on cardiopulmonary bypass with heart rate and aortic pressure maintained constant.

Numerous studies have failed to find that diazepam produced any significant alteration in the functions of the heart. Katz *et al.* (14), studying the effects of tilting on the cardiovascular system of healthy adult male volunteers, could find no changes in the blood pressure or pulse rate in doses producing sedation of various degrees. Prindle and co-workers (16) were unable to demonstrate any significant changes in contractility on the isolated cat papillary muscle preparation as a result of diazepam studied over a wide range of concentrations.

Others have demonstrated depressant effects in the various indices of cardiac performance, in varying degree. Starley and Michie (17) administered diazepam to conscious dogs and found only minimal alterations in cardiovascular function when compared to animals receiving a placebo. In patients undergoing cardiac catheterization, whose control cardiac indices were below normal, Dalen and associates (18) found that diazepam (5–10 mg) given intravenously caused no significant changes in pulmonary arterial pressure, heart rate, stroke volume, pulmonary vascular resistance, or systemic vascular resistance. However, in patients whose control cardiac indices were in the high-normal range, the cardiac index decreased significantly, although the observed changes in blood pressure were without clinical correlates and did not require therapy. Chai and Wang (19) demonstrated a slight reduction of the resting blood pressure, heart rate and cardiac contractile force in the anesthetized cat as a result of administering diazepam intravenously (0.1 mg/kg).

A report by Bianco and co-workers (20) stated that the diazepam solution as available, rather than the diazepam itself, resulted in a transient depression of ventricular performance when given as a bolus into the pulmonary artery of canine right heart bypass preparations. This occurred in doses higher than those ordinarily used for cardioversion. They attributed these effects to the vehicle in which diazepam was contained and not to the drug itself.

In the present experiments, the effects of diazepam were observed in the noninnervated 4-day-old embryonic heart as well as in the innervated 7-day-old embryonic heart of the chick. The interaction of diazepam with other selected compounds was also studied in an attempt to determine the mechanism of the cardiac response to diazepam. In addition, the vehicle used in the injectable commercial preparation was studied.

Methods. The heart of the chick embryo was used in all studies. The source and handling of eggs and determination of the ages of chick embryos have been previously described (21). The apparatus and method of recording heart rate and contractile amplitude isotonically have been previously reported (22). All studies were done on both 4- and 7-day-old embryonic hearts in order to determine if innervation could be a factor in the response to diazepam.

[1] This work was partially supported by NIH Research Training Grant No. GM-01213.

For the cumulative dose–response curves, diazepam was added and the heart rate and contractile amplitude determined after 3 min. Diazepam was again added to bring the concentration to the desired level and the heart rate and contractile amplitude determined after an additional 3 min etc. For the remaining studies, the first test compound was added and the heart rate and contractile amplitude determined after 3 min. The second test compound was then added and these two parameters measured after 3 more minutes. The means and standard errors of identical samples were calculated for both the heart rate and the amplitude of contraction. Significance levels were determined using the appropriate Student's *t* test.

The compounds tested were as follows: Diazepam injectable (preparation of Valium), and the vehicle for diazepam (Roche Laboratories); atropine sulfate (Merck); l-norepinephrine *d*-bitartrate (Calbiochem).

Results. The effect on the embryonic chick heart of increasing the concentration of diazepam in the bathing medium is shown in Figs. 1 and 2. The 4-day-old embryonic hearts were slightly more sensitive to the depressant effects of the drug than were the 7-day-old embryonic hearts. Onset of the depressant effects was rapid, becoming nearly maximal during the first half-minute after addition of diazepam. Because of this rapid development of maximal response, the 3-min time interval between successive additions of drug was chosen in order that the cumulative dose–response curve could be completed within a reasonable length of time.

The average concentration of diazepam in the bathing medium found to reduce the heart rate and contractile amplitude to 50% of the control value was approximately 1.0×10^{-4} *M* for the 4-day-old hearts and approximately 1.6×10^{-4} *M* for the 7-day-old hearts. Concentrations of diazepam lower than 1.0×10^{-5} *M* had no detectable effects on the heart rate or amplitude of contraction, while 2.0×10^{-4} *M* or greater caused cardiac standstill in diastole. The diazepam vehicle had no detectable effects on the embryonic hearts by use of that amount of vehicle necessary to attain a drug concentration of 1.0×10^{-4} *M*.

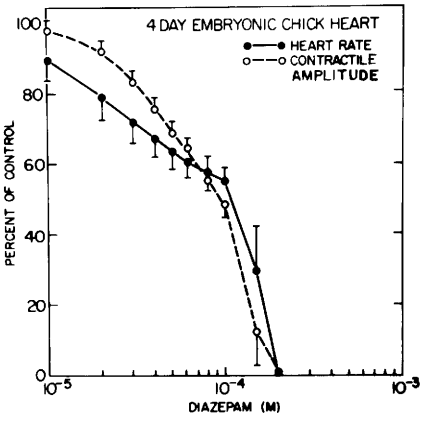

FIG. 1. Cumulative dose–response curve of diazepam effect on the heart rate and contractile amplitude of 4-day-old embryonic chick hearts, showing the mean and standard error of four experiments.

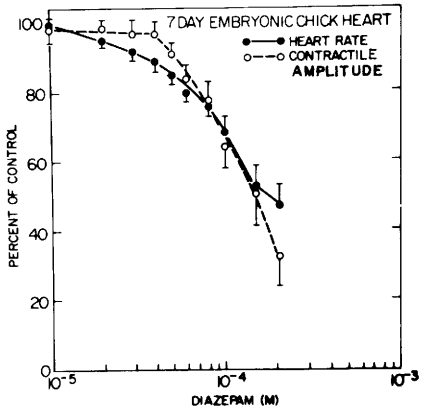

FIG. 2. Cumulative dose–response curve of diazepam effect on the heart rate and contractile amplitude of the 7-day-old embryonic chick hearts, showing the mean and standard error of four experiments.

In studies not shown, the depression of the heart rate and amplitude of contraction, caused by diazepam (6×10^{-5} *M*) in either 4- or 7-day-old hearts was not prevented by prior addition of atropine (1.4×10^{-6} *M*) to the bath. Similarly, the depression due to diazepam was not reversed by subsequent exposure to atropine in either group.

By contrast, in both 4- and 7-day-old embryonic hearts depressed by diazepam (6×10^{-5} *M*), norepinephrine (2.0×10^{-6} *M*) was able to increase the contractile amplitude to levels slightly greater than the predrug control values. However, norepi-

nephrine was unable to reverse the depression of the heart rate.

Discussion. Extensive studies by previous investigators (23, 24) have established that innervation of the embryonic chick heart occurs only after the 4th day of incubation. Diazepam depressed both the rate and amplitude of contraction on the isolated embryonic hearts from both the 4- and 7-day-old preparations. The degree of depression in these two parameters was directly related to the concentration of diazepam in the bathing fluid.

Foster and Frings (25) reported that the highest concentration of diazepam in the blood resulting from a continued daily dosage of 30 mg/day was 100 μg/100 ml. This corresponds to a concentration of about 3.5×10^{-6} *M*. This concentration in the present study had no detectable depressant effects on the embryonic hearts. However, the problem of extrapolation of data from one species to another and from embryonic to adult tissue is well recognized. Also, the concentration which totally arrested the embryonic hearts, 2.0×10^{-4} *M*, would not be reached clinically unless the drug were administered directly into the heart or vena cava, such as accidently through a central venous pressure catheter etc.

The use of a concentration of atropine sufficient to block the negative inotropic and chronotropic effects of 10^{-7} g/ml of acetyl choline (26) did not change the response of the embryonic hearts to diazepam; thus stimulation of cholinergic receptors did not play a role in the depression. That norepinephrine was able significantly to enhance contractile amplitude in the moderately depressed hearts indicates that these depressed hearts are still responsive to adrenergic agonists. The vehicle by itself had no effect. In this respect, the present results are in disagreement with those of Bianco *et al.* (20). Thus it is possible that the depression caused by diazepam was due to a direct effect on the myocardium.

Summary. Diazepam decreased the rate and amplitude of contraction in isolated embryonic chick hearts in a dose-dependent manner in both the noninnervated hearts obtained from 4-day-old embryos and the innervated hearts from 7-day-old embryos. The concentration of diazepam necessary to reduce the heart rate and contractile amplitude to 50% of the control values was about 1×10^{-4} *M*. Concentrations less than 1.0×10^{-5} *M* had no detectable depressant effects. Prior administration of atropine did not alter the depression induced by diazepam. Norepinephrine was able to stimulate the amplitude of contraction in the diazepam-depressed heart while atropine was without effect. The vehicle used in the clinical injectable preparation of diazepam had no depressant effects. The mechanism of action of the diazepam-induced depression on the isolated embryonic chick heart may be a direct depression of the myocardium.

1. Stiles, E. M., Pugh, D. M., and Dunn, M., J. Kans. Med. Soc. **69**, 277 (1968).
2. Turkell, R. A., and Lemmert, W. A., S. Med. J. **62**, 61 (1969).
3. Barrett, J. S., and Hey, E. B., *J. Amer. Med. Ass.* **214**, 1323 (1970).
4. Rosenblatt, W. H. and Nettles, D. C., Miss. State Med. Ass. J. **11**, 57 (1970).
5. Rotem, C. E., Can. Med. Ass. J. **103**, 1381 (1970).
6. Lyons, S. M., and Clark, R. S. J., Brit. Med. J. **4**, 229 (1971).
7. Nevins, M. A., J. Amer. Med. Ass. **215**, 643 (1971).
8. Somers, K., Gunstone, R. F., Patel, S. K., and D'Arbela, P. G., Brit. Med. J. **4**, 13 (1971).
9. Bell, D. S., Brit. Med. J. **1**, 159 (1969).
10. Viala, A., Cano, J. P., Dravet, C., Tassinari, C. A., and Roger, J., Psychiat. Neurol. Neurochir. **74**, 153 (1971).
11. Stovner, I., and Endresen, R., Anaesthesist **18**, 242 (1969).
12. Knapp, R. B., and Dubow, H., Anesth. Analg. **49**, 722 (1970).
13. Knapp, R. B., and Dubow, H., S. Med. J. **63**, 1451 (1970).
14. Katz, J., Finestone, S. C., and Pappas, M. T., Anesth. Analg. **46**, 243 (1967).
15. Abel, R. M., Staroscik, R. N., and Reis, R. L., J. Pharmacol. Exp. Ther. **173**, 364 (1970).
16. Prindle, K. H., Gold, H. K., Cardon, P. V., and Epstein, S. E., J. Pharmacol. Exp. Ther. **173**, 133 (1970).
17. Starley, J. W., and Michie, D. D., Curr. Ther. Res. Clin. Exp. **11**, 796 (1969).
18. Dalen, J. E., Evans, C. L., Banas, J. S., Brooks,

H. L., Paraskos, J. A., and Dexter, L., Anesthesiology **30,** 259 (1969).
19. Chai, C. Y., and Wang, S. C., J. Pharmacol. Exp. Ther. **154,** 271 (1966).
20. Bianco, J. A., Shanahan, E. A., Ostheimer, G. W., Guyton, R. A., Powell, W. J., and Daggett, W. M., J. Thorac. Cardiovasc. Surg. **62,** 125 (1971).
21. Ignarro, L. J., and Shideman, F. E., J. Pharmacol. Exp. Ther. **159,** 29 (1968).
22. Berry, D. G., Anesth. Analg. **53,** 919 (1974).
23. Hamilton, H. L., "Lillie's Development of the Chick," 3rd ed. Holt, Rinehart and Winston, New York (1952).
24. Romanoff, A. L., "The Avian Embryo." Macmillan, New York (1960).
25. Foster, L. B. and Frings, C. S., Clin. Chem. **16,** 177 (1970).
26. McCarty, L. P., Ph.D. thesis. University of Minnesota, Minneapolis (1960).

Received January 6, 1975. P.S.E.B.M. 1975, Vol. 150.