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PREPARATION OF THE DOG

JOSEPH M. BENFORADO AND PETER N. WITT

Department of Pharmacology, Harvard Medical School, Boston 15, Massachusetts

THE INFLUENCE OF HEART RATE UPON THE LETHAL DOSE OF VERATRIDINE IN THE HEART-LUNG PREPARATION OF THE DOG¹

JOSEPH M. BENFORADO² AND PETER N. WITT³

Department of Pharmacology, Harvard Medical School, Boston 15, Massachusetts

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When certain drugs are administered by continuous infusion into the heart-lung preparation of the dog, and when the lethal dose is determined by calculating the total amount of drug infused up to the point of ventricular fibrillation, two factors are known to influence the lethal dose: the rate of infusion and the weight of the heart.

As the rate of infusion, starting at high rates, is reduced, the lethal dose decreases progressively until at very low rates it tends to become constant. This constant value has been designated by Farah (1946a) as the minimal lethal dose since this dose cannot be reduced by further decrease in the rate of administration. The characteristic highest rate of administration at which the minimal lethal dose can still be obtained has been called the optimal rate of administration (Farah, 1946a).

There is some controversy concerning the measurement of the weight of the heart. Heart weight in a heart-lung preparation changes with the duration of the experiment since edema accumulation in the heart increases with the lengthening of the experimental time. The actual heart weight at the conclusion of an experiment represents initial heart weight plus edema fluid. Various methods of correcting to true heart weight give widely different values. In the present investigation we have chosen for the correction of the heart weight the body weight/heart weight ratio (Wood and Moe, 1942).

That the above mentioned factors influence the lethal dose has been demonstrated by Farah in the heart-lung preparation for cardiac glycosides (1946a, 1946b, 1947, 1948, 1953), and the same seems to be true for veratridine (Benforado, 1953). However, the coefficient of variation in the case of the veratridine experiments was so large that additional factors were suspected to influence the lethal dose.

The experiments to be reported followed from the observation that hearts which showed an early atrio-ventricular block, or an early sinus bradycardia, during veratridine infusion—and as a result continued beating at a low rate—seemed to survive relatively long and went into fibrillation only after the infusion of a relatively high dose. On the other hand, hearts in which these arrhythmias occurred late during infusion, or did not occur at all, fibrillated when

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² William F. Milton Research Fellow in Pharmacology.

³ Rockefeller Travelling Fellow.

the dose infused was still low. This led us to explore the influence of heart rate on lethal dose at a constant rate of infusion.

METHOD. The dog heart-lung preparation was set up as described by Krayer (1931). Mongrel dogs of either sex weighing between 8.7 and 12 kgm. were used. The artificial resistance was set to give a mean arterial pressure of 100 to 110 mm. of mercury. The right atrial pressure was recorded with a water manometer; and in some experiments pulmonary arterial pressure was recorded with a bromoform manometer. Systemic output was measured with a Weese Stromuhr and initially was set between 600 and 750 ml. per minute. The blood volume varied between 850 and 1000 ml. at the start of the experiment, and the blood temperature was kept between 37.7° and 39° C. Heart rates were counted at least every two minutes by means of an inkwriting electroencephalograph used as an electrocardiograph. The endpoint of the experiment was either ventricular fibrillation as recorded by this machine, or a systemic output of less than 50 ml. per minute. The latter endpoint enabled us to include experiments with hearts that beat finally at very low rates and never spontaneously fibrillated. These hearts survived extremely long and the respective experiments would probably have been eliminated by other authors. Since we were interested only in the effect of heart rate, we included them with our other results, thus appreciably extending the range of heart rates.

The veratridine used was the free base prepared from veratrine⁴ by Deliwala and Kupchan. It had a rotation of $[\alpha]_D^{25} + 6^\circ$ (c. 2.00 in ethanol). Stock solutions containing 1 mgm. per ml. were prepared by dissolving the alkaloid in distilled water with an equimolar amount of hydrochloric acid. For infusion the veratridine solution was prepared freshly by diluting the stock solution with distilled water to the desired concentration of 480 microgm. per ml. The infusion was made into the venous supply cannula at a constant rate by means of a syringe with a motor driven plunger. The syringe was set to deliver 0.0208 ml./minute, which corresponds to an infusion rate of 10 microgm. per minute. In preliminary experiments it had been determined that this rate of infusion represented the optimal rate of administration according to the definition of Farah (1946a) and gave the minimal lethal dose.

In addition to the ordinary range of heart rate some hearts were stimulated at a rate of 190 to 200 stimuli per minute. For this purpose a Grass stimulator was used, delivering electrical square waves of 8 volts and 1 millisecond duration. The stimulus was transmitted to the right atrium through small electrode clamps clipped to the muscle tissue. The intensity of the stimulus was supramaximal.

The heart weight was determined by multiplying the body weight of the dog by a factor of 0.000763. This is an empirical figure found by Wood and Moe (1942) relating heart weight to body weight in the weight range of our dogs.

RESULTS. The results of all our experiments are shown in table 1, in which the experiments are grouped in order of increasing average rate. In this sequence the dates are in approximately random order. Column one, which gives the experimental times, permits the calculation of the amount of veratridine infused, since the rate of infusion for all experiments was 10 microgm. per minute. Experimental time multiplied by 10 gives the infused amount of drug in microgm. The total numbers of beats were obtained from the electrocardiogram taken at least every two minutes as described. Total number of beats divided by experimental time in minutes yields the number of beats per minute which is called average rate. The lethal dose in column 4 is the amount of veratridine infused in microgm. divided by the heart weight.

⁴ The veratrine sample was generously supplied by S. B. Penick & Company, New York, N. Y.

TABLE 1

The influence of heart rate upon lethal dose of veratridine in the heart-lung preparation of the dog

DATE	1 EXP. TIME min.	2 TOTAL NO. OF BEATS	3 AVERAGE RATE beats/min.	4 LETHAL DOSE microgm./gm. heart
Mar. 18* †	300	29,500	98	39.3
July 15†	280	27,907	100	42.2
Dec. 22†	282	28,728	102	35.2
May 15	222	23,651	107	29.1
June 1	149	16,678	112	16.4
May 20* †	240	27,514	115	26.9
Dec. 19†	196	24,618	126	29.5
Dec. 17	165	23,679	144	18.2
May 13*	114	18,858	165	14.7
Mar. 25*	105	19,760	188	12.9
Mar. 4*	118	22,420	190	16.6
Mar. 20*	110	21,060	191	12.4
May 22*	80	15,721	197	8.7
Coefficient of variation:	42.5%	19.7%	28.3%	47.8%

* Heart driven by stimulator.

† Heart did not fibrillate spontaneously.

The coefficient of variation at the bottom of each column indicates the high variation of the lethal dose when only the rate of infusion and the heart rate are taken into consideration. The total number of beats, on the other hand, is the factor that varies least in our experiments.

As can be seen from the table, average rates increase while lethal doses decrease. Calculating the linear regression of lethal dose on average rate a regression coefficient $b^5 = -0.24$ was found. The regression line is shown in figure 1. Each experiment is represented by a single point. To test if the regression coefficient is significantly different from zero (i.e. the coefficient which would be expected if the lethal dose was not influenced by heart rate) the coefficient of correlation r^6 was calculated. Its value $r: 0.8580$ is significant at the 1 per cent probability level, $P 0.01: 0.6835$. The slope of our regression line is therefore significantly different from zero.

DISCUSSION. The results of our experiments indicate that the lethal dose of veratridine, under the conditions of our experiments, is dependent upon heart rate as well as upon rate of infusion and weight of the heart. Since the lethal dose at the optimal rate of infusion varies with the heart rate, the term minimal lethal dose of veratridine will have meaning only when the heart rate at which it was obtained is specified.

$$^5b: \frac{\sum x \cdot y - \bar{x} \cdot \sum y}{\sum x^2 - \bar{x} \cdot \sum x}$$

(Mainland, 1952)

$$^6r: \frac{\sum (x - \bar{x}) \cdot (y - \bar{y})}{\sqrt{\sum (x - \bar{x})^2 \cdot \sum (y - \bar{y})^2}}$$

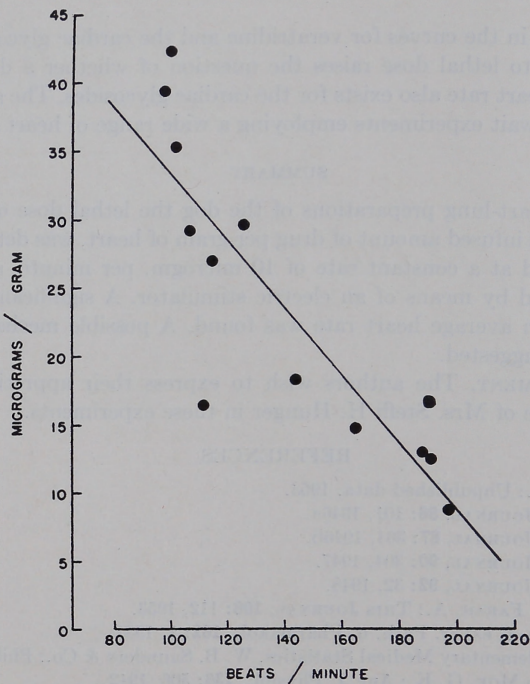


FIG. 1. The influence of heart rate upon the lethal dose of veratridine. Each point represents one experiment; the line shows the linear regression of lethal dose/gram heart weight on average rate with a regression coefficient $b:-0.24$. For further explanations see text.

The lethal dose, as measured, actually consists of three fractions the values of which are unknown. At the termination of an experiment some drug may be left in the blood, and of the amount taken up by the heart only a fraction may actually have killed the heart. The fact that there exists a minimal lethal dose and an optimal rate of administration for veratridine, as well as for the cardiac glycosides, suggests that all of the infused drug is taken up by the heart. Since in our experiments a rate of administration comparable to Farah's optimal rate of administration was used, it might be assumed that the fraction of drug actually killing the heart is identical with, or is in constant relation to, the lethal dose. On this basis the decrease of lethal dose with increasing heart rate might be accounted for by an increase in sensitivity of the heart to veratridine.

Since in our experiments the total number of beats is relatively constant, it is likely that veratridine acts at a metabolic level that is in some manner related to the heart beat, each beat representing a fraction of the total metabolic effect required for the heart to be killed. The fast beating heart makes available for drug action more fractions per unit time of this total requirement than does the slow beating heart and so arrives at the total or lethal effect sooner having required less drug (lower lethal dose) in the process.

The similarity in the curves for veratridine and the cardiac glycosides relating rate of infusion to lethal dose raises the question of whether a dependency of lethal dose on heart rate also exists for the cardiac glycosides. The answer to this question must await experiments employing a wide range of heart rates.

SUMMARY

In thirteen heart-lung preparations of the dog the lethal dose of veratridine, calculated as the infused amount of drug per gram of heart, was determined. The drug was infused at a constant rate of 10 microgm. per minute, and the heart rates were varied by means of an electric stimulator. A significant dependency of lethal dose on average heart rate was found. A possible mechanism for this dependency is suggested.

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