Effect of Length of a Ryanodine-treated Muscle on Oxygen Uptake. (18111)

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In muscle physiology there are many factors which are related to the length of the muscle. To mention just a few, there are: birefringence(1), maintenance heat(2), shortening heat(3), tension(4), and oxygen consumption of a resting muscle after stretching (5,6). Evidence is given in this paper to show that the length also plays an important part in energy release in the muscle treated with Ryanodine even though excitation and mechanical work have been reduced to a minimum.

Methods. Frog sartorious muscles were dissected out and allowed to equilibrate in Ringer's-phosphate solution for 18 to 24 hours at 4°C. Ryanodine, in a final concentration of 1 x 10^{-5}, was introduced under two different conditions. In the first case, Ryanodine was introduced from a side-arm of a Warburg flask in the usual manner. The Ryanodine was dumped into the solution containing the muscle after a normal period of one hour which had followed an appropriate equilibration period of 20 minutes.

The second method of introduction of the Ryanodine was designed to allow for a more even distribution of the drug throughout the muscle. This was accomplished by cooling to 4°C the Warburg flasks containing the muscle and Ringer's as well as the Ryanodine solution before dumping the drug. A 30-minute interval was allowed for diffusion of the drug through the muscle before the temperature was raised. The action of Ryanodine resumed its normal course after it was brought back to the experimental temperature (23°C). More regular and more even contractures were produced by this procedure.

In the later experiments the muscle length was measured by locating the ends of the muscle with calipers and measuring the distance between the points on a millimeter scale. The rest length of the muscle was taken by measuring the length of the muscle in the animal when the leg was extended.

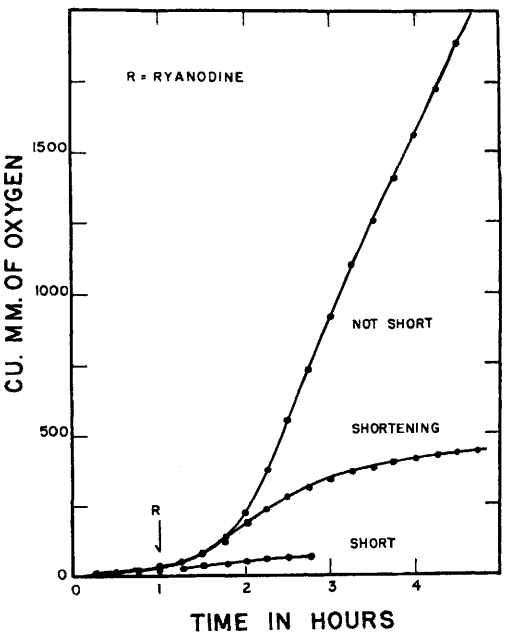

FIG. 1.

Comparison of total oxygen consumption of shortened and unshortened Ryanodine-treated frog muscle. Final concentration of Ryanodine in all 3 curves was one part in 100,000. The top curve represents 8 experiments in which the muscles had not shortened noticeably at the end of the experiment. The middle curve represents 9 experiments in which the muscles had shortened markedly by the end of the experiment. In the curve marked "short" the muscles in 8 experiments were allowed to shorten first and then the oxygen consumption was measured for the period indicated. The arrow marked "R" does not apply for this curve.

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Shortening was calculated as per cent of rest length.

Results. During a study of the action of inhibitors on Ryanodine-treated muscles it was observed that the shortened muscles had a lower oxygen consumption than did the longer ones, regardless of the inhibitors used. In the first figure the total oxygen consumption of the muscle has been plotted against time. The 3 curves represent averaged data from 3 different groups of experiments. The first curve represents the average oxygen consumption of 8 experiments in which the muscles had not shortened noticeably. In the second curve are the averaged results from 9 experiments including all the muscles which had shortened and were classed as markedly shortened at the conclusion of the experiment. In both these groups Ryanodine alone had been introduced from the side arm after a normal resting level had been established for one hour. In the third curve, representing the averaged results of eight experiments, the muscles were allowed to shorten first and then the oxygen level was measured. These curves clearly indicate that there is a marked difference between the oxygen consumption of the unshortened muscles as compared to the contracted muscles.

In the second figure is given the oxygen consumption of one muscle as it gradually contracted. The measurements of length were made by observing the muscle through the glass flask and are therefore somewhat inaccurate. Even so, this curve indicates that there is a gradual decrease in oxygen consumption as the muscle shortens. Most muscles do not shorten this slowly under the influence of Ryanodine so this type of analysis can not always be made.

In the last phase of this work (Fig. 3), an attempt has been made to correlate the oxygen consumption with the length of the muscle. The length reported was the length of the muscle as measured at the end of the experiment. Thus each point in this figure represents one complete experiment.

In addition the length of the muscle in about half of the experiments was estimated through the flask after every reading of the manometer. These observations were useful

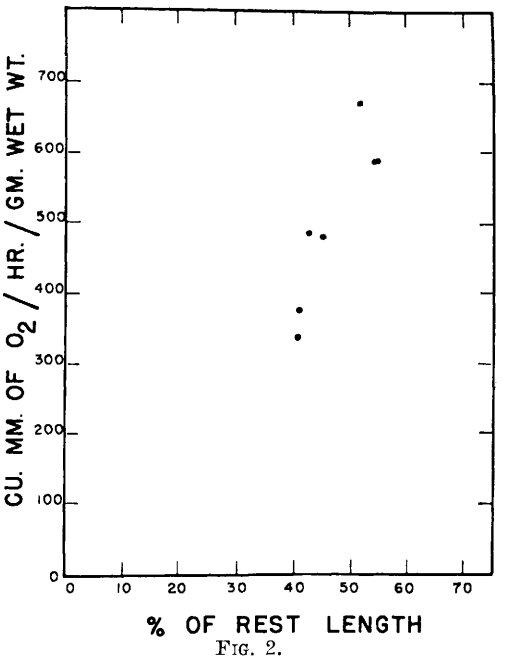

FIG. 2.

Effect of length on oxygen consumption as measured by one muscle. The final concentration of Ryanodine was one part in 100,000. The length was measured in the flask as the oxygen consumption was measured.

in determining when most of the shortening had taken place; however, they are not included in the graph.

In general the shortening of the muscle takes place very rapidly once it starts. The oxygen consumption was taken over that part of the oxygen curve where the oxygen requirement had leveled off, immediately after the muscle had stopped shortening. In order to determine this value, the rate of oxygen consumption was plotted against time for each determination. These curves are not shown in the text.

In those experiments in which the lengths were not estimated after each reading of the oxygen uptake, the final oxygen uptake for a given length was taken after the curve had leveled off, following the initial oxygen spurt at the beginning of the contracture. This oxygen consumption was the average for this level portion of the curve and represents several oxygen measurements.

For each muscle length down to the 30% of rest length, there is a corresponding oxygen consumption when the muscles have been

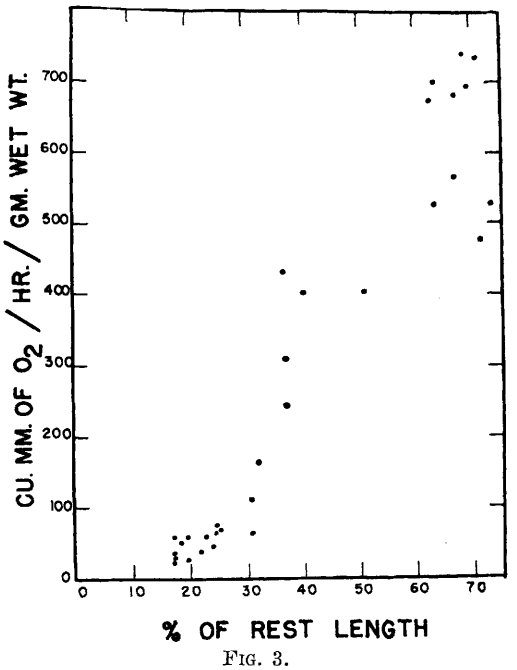

FIG. 3.

Effect of length on oxygen consumption as measured by separate muscle experiments. The length of the muscle was measured at the end of the experiment using calipers. One part in 100,000 was the final concentration of Ryanodine.

treated with the same concentration of Ryanodine. Below 30% of the rest length the oxygen consumption has fallen to at least the resting metabolism level and remains at this level or less with still shorter muscles.

Discussion. The action of Ryanodine is an interesting one. This drug releases a large amount of energy in the muscle even though the muscle is unable to do any appreciable mechanical work or to be stimulated electrically. G. A. Edwards and co-workers(7) have demonstrated that the excitation process is completely blocked by Ryanodine. In repeating part of this work, we found that the blocking of excitation occurred well before the contracture developed. In the present work a much higher increase in the oxygen consumption of frog muscle was found with Ryanodine-treated muscles than was reported by Edwards and co-workers(7).

In those instances in which the drug was allowed to diffuse evenly into the muscle at low temperature before being brought to room temperature the muscle fibers in most cases contracted in perfect alignment if allowed to shorten under no load. However, if any restraining force were used, compelling the muscle to develop tension as it shortened, then considerable damage was done to the fibers.

Many investigators have worked on contractures. Contractures in general have at least one advantage over the simple twitch in the study of muscle physiology. The contracture develops over a longer period of time than a simple twitch and thus allows for a measurement of energy as well as for oxygen consumption during the period of shortening.

Since under the conditions in which Ryanodine failed to cause a marked shortening or contracture the oxygen consumption remained extraordinarily high and only diminished with contracture or shortening, it is believed that it is the shortening and not the drug which causes the final lowering of the oxygen consumption. It thus appears that the length of the muscle regulates any excess oxygen consumption above the resting level. Other contractures probably act in a similar manner to that of the Ryanodine-produced contracture. For example, caffeine increases the oxygen consumption of muscle to almost the same magnitude as does Ryanodine. In addition, a higher concentration of caffeine produces contracture. Thus contracture and subcontracture levels are mentioned in the literature. More consistently higher oxygen uptakes are found with concentrations slightly below the contracture level than above it(8). The authors of this present investigation would like to suggest that this mechanism (produced by Ryanodine) exists also in other forms of contracture.

As judged by the oxygen consumption shown in Fig. 3, it can be deduced that the length of the muscle controls the release of energy within the muscle. It appears that the energy is first released in certain fixed amounts, depending upon the stimulating condition or drug and then the energy is further

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controlled by the muscle length. More exact methods of recording length and oxygen consumption simultaneously will be devised to improve the accuracy of the curve showing this relationship. This curve should also be very useful in determining other theoretical energy calculations involved in muscle physiology.

Conclusions. Ryanodine causes a high oxygen consumption in muscles which do not shorten. In those muscles that shorten the oxygen consumption falls off as the muscle shortens. The length of the muscle seems to regulate the oxygen consumption of the Ryanodine-treated muscle. Complete cutting off of the extra metabolism due to Ryanodine occurs at 30% of the rest length.

We are indebted to Dr. Roeder and to Merck and Co. for supplying us with the purified Ryanodine powder.

We wish to thank Dr. Robert W. Ramsey for his helpful advice and interest in this problem. We are also indebted to him for making many of the microscopic observations on the structure of the muscle at the conclusion of the experiments.

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Pharmacology of Para-substituted Derivatives of Diphenhydramine. (18112)

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Among a number of the derivatives of 2-benzhydryloxy-N-N-dimethylethylamine (diphenhydramine), tested for their antihistaminic activity, the para-substituted compounds were found of special interest from the standpoint of the relationship between chemical constitution and pharmacological action. In this report, a comparison of the pharmacological properties will be made of the para-alkyl-, para-methoxy- and the para-halogen-derivatives of 2-benzhydryloxy-N, N-dimethylethylamine with a view of appraising their therapeutic applicability.

Materials and methods. The following compounds,* p-methyl, p-ethyl, p-n-propyl, p-methoxy, p-fluoro, p-chloro, p-bromo and p-iodo benzhydryloxy-N, N-dimethylethylamine as their hydrochlorides, have been investigated. They are white crystalline compounds, freely soluble in water.

The antihistamine potency of a compound was determined: (a) by protection against the lethal toxicity of histamine aerosol in guinea pigs, and (b) by suppression of the histamine-induced contraction of an isolated intestinal strip with procedures as previously described(1,2). Similar technics were employed for estimating anticholinergic and myotropic spasmolytic activities. Acetyl-beta-methylcholine chloride (methacholine) was used to produce a fatal cholinergic bronchospasm to guinea pigs(3). At least 48 animals, 12 per group for each dose, were used to determine a 50% protective dose of the drug.

Acute toxicity was determined in mice by intraperitoneal injection. The LD_{50} and average standard error were calculated from the mortalities, obtained with 5 doses of the drug in groups of 20 animals each, by the method of Miller and Tainter(4).

The potentiation of epinephrine by and the atropine-like action of these compounds on blood pressure were investigated in dogs under pentobarbital anesthesia with standard procedures.

Results and discussion. As the data in

* Kindly made available to us by Drs. R. W. Fleming and G. Rieveschl, Chemical Division Research Laboratories, Parke, Davis and Co.

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