

EFFECTS OF VERAPAMIL ON CHICKEN BIVENTER - CERVICIS MUSCLE

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Abstract - Verapamil produces a sustained contraction in isolated biventer-cervicis muscle of chickens between 2-8 days old. From cumulative dose-response curves, ED₅₀ of $1.57 \times 10^{-4} M$ was calculated for this effect of verapamil. When isolated chicken biventer-cervicis muscle was electrically stimulated, verapamil $3.67 \times 10^{-4} M$ had no effect on twitch contractures but increased the base line tone of the muscle. Glycerol treatment of the muscle reduced the responses to acetylcholine and KCl but had little effect on contracture produced by verapamil, and no effect on contracture produced by caffeine. Incubation of the muscles with calcium-free Krebs solution omitted the responses of the muscle to acetylcholine and reduced the response to caffeine. Again, the responses to caffeine and verapamil were less affected compared to KCl. Addition of ethylene glycol tetra-acetic acid (EGTA) (2.5 mM) abolished the responses of muscle to all compounds. It was concluded that verapamil produces contracture of the muscle by release of calcium from intracellular stores.

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Key words: Verapamil, glycerol, biventer-cervicis muscle, chicken

INTRODUCTION

During the past decades evidence has been obtained of the existence of specific channels for calcium ions that activate the contractile machinery (1,2). Therefore, the discovery of the new family of highly potent organic calcium antagonists which are capable of specifically blocking these channels has opened new horizons in understanding the nature of these channels and provided new approaches to treatment of various diseases (1). In addition, the involvement of dihydropyridine receptors in excitation contraction coupling in skeletal muscle has been reported (3). On the other hand, it has been reported that dihydropyridine receptor controls

T-tubule depolarization-induced sarcoplasmic reticulum calcium release and calcium-dependent mechanisms in cell hemogenates from rabbit skeletal muscle (4). In this study the effect of verapamil on twitch contractures of biventer-cervicis muscle of chickens was investigated.

MATERIALS AND METHODS

Chickens (2-8 days old) were anesthetized with sodium phenobarbitone (9% solution in water, 0.2 ml/100 g) injected into a wing vein. Biventer-cervicis muscle and its tendon which encapsulates the motor nerve was dissected and assembled according to the method described by Ginsborg and Warriner in 1960 (5). The organ bath (15 ml total capacity containing krebs solution) was maintained at a constant temperature (37°C) and oxygenated with 95% oxygen and 5% carbon dioxide mixture. Solutions of verapamil were added in a cumulative manner to the incubation media and contractions of the muscles were recorded isotonicly with Narco Isotonic Myograph Transducer and Narco physiograph (DMP-4A). The muscles were electrically stimulated at 0.1 Hz, 1 msec pulse duration and supramaximal voltage, and the twitch contractions were recorded.

The contractile responses of the chicken biventer-cervicis muscles to acetylcholine (2.5×10^{-4}), KCl (10mM), caffeine (5mM) and verapamil ($3.67 \times 10^{-4} M$) were recorded. The muscles were then incubated with Krebs solution

containing glycerol (840mM) for 1-1.5 hour, with several washes after which the muscles were returned to normal Krebs solution (6,7). Responses of the muscles after glycerol treatment to acetylcholine, KCl, caffeine and verapamil were recorded.

Effect of Calcium-Free Incubation Media

The responses of the muscles to acetylcholine ($2.5 \times 10^{-4}M$), KCl (10 mM), caffeine (5 mM) and verapamil ($3.67 \times 10^{-4}M$) were recorded. Then muscles were incubated either in calcium-free Krebs solution or calcium-free Krebs solution containing 2.5 mM ethylene glycol tetra-acetic acid (EGTA) for one hour with several washes. The responses of the muscles in the modified media to above mentioned agents were recorded.

Drugs

All drugs were obtained from Sigma Co.

Statistical analysis

Data presented are given as mean \pm S.E.M, and significances have been tested by Student's t-test. Levels of significance are denoted by * $p < 0.05$ and ** $p < 0.01$.

RESULTS

Verapamil produces a dose-dependent, sustained contraction in chicken biventer-cervicis muscle (Fig. 1). However, this effect is reversible and the several washes the muscle slowly returns to relaxed condition.

The ED₅₀ for contracture effect of verapamil was calculated from log dose-response curve (Fig. 1) and was found to be $1.57 \times 10^{-4} \pm 0.225 M$. Although this value seems excessively high, it is in consistence with the value reported (8). When the isolated muscle was electrically stimulated, verapamil up to $3.67 \times 10^{-4}M$ had no effect on the magnitude of twitch contracture but increased the baseline tone of the muscle (Fig. 2). The glycerol-treated muscles were unresponsive to

potassium and acetylcholine without affecting the sensitivity to caffeine (Fig. 3). In addition, a slight reduction in the response to verapamil was

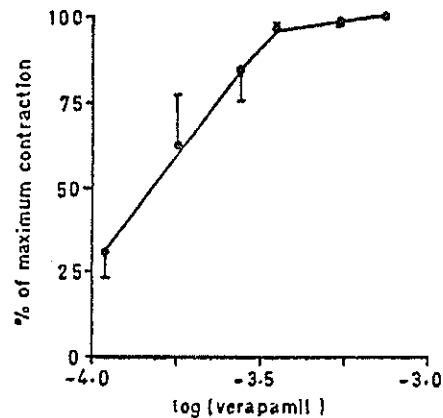


Fig. 1. Effect of different doses of verapamil on maximum contraction in chicken biventer-cervicis muscle.

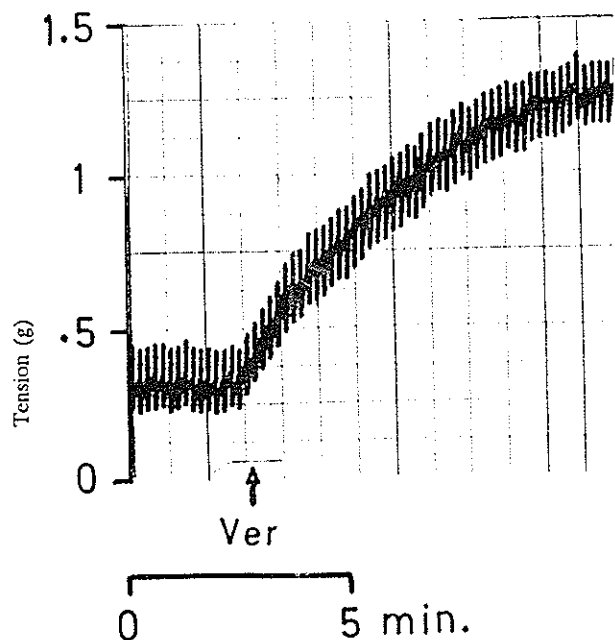


Fig. 2. The isolated muscle was electrically stimulated. verapamil up to ($3.67 \times 10^{-4} M$) had no effect on the magnitude of twitch contracture but increased the baseline tone of the muscle.

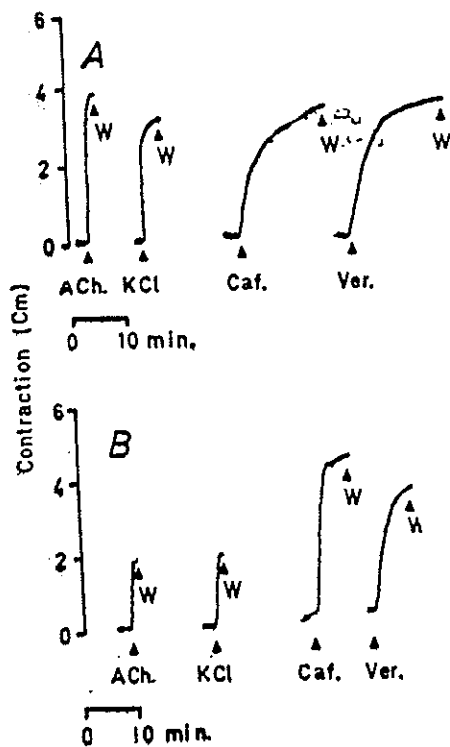


Fig. 3. The effect of glycerol treatment on contraction of biventer-cervicis muscle of chicken. Part A: The control responses of muscle to different agonists. Part B: The responses of the same muscle after glycerol treatment to the above mentioned agonists. The Y axis indicates the amplified change in the length of tissue.

observed. When muscles were treated for one hour with calcium-free Krebs solution and then tested for contracture ability with the above mentioned drugs, it was found that response to acetylcholine was reduced almost to zero. There was also a substantial reduction in the response to KCl (13.23 % of control). However, the response of the muscles to verapamil and caffeine was less affected (41.3 % and 71.4 % of control respectively) (Fig. 4B).

However, in muscles treated with calcium-free Krebs solution containing EGTA (2.5 mM), the responses of the muscles to all four drugs were abolished (Fig. 4C).

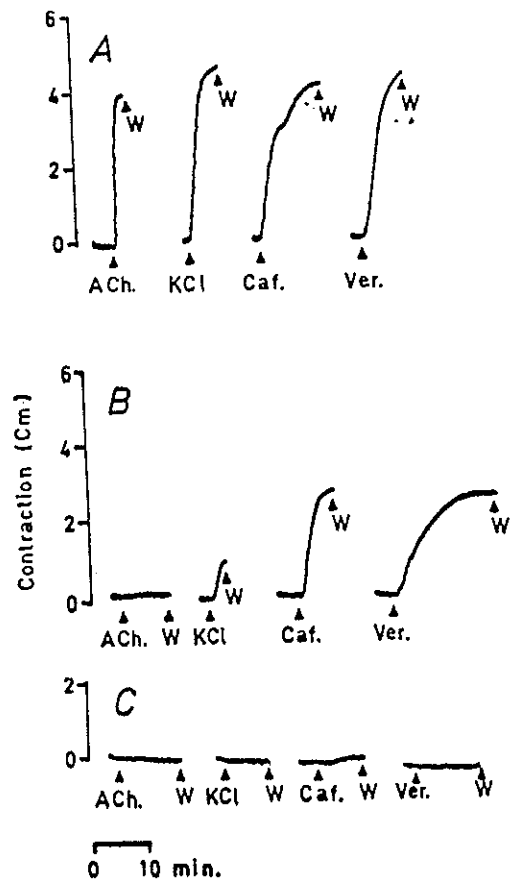


Fig 4. The effect of Ca-free incubation media on contraction of biventer-cervicis muscle of chicken. Part A: The control responses of the muscle to different agonists. Part B: The responses of the muscle in the ca-free Krebs solution to the above mentioned agonists. Part C: The responses of the muscle in the ca-free Krebs solution containing EGTA, 2.5 mM. The Y. axis indicates the amplified change in the length of tissue.

DISCUSSION

These data clearly demonstrate that, verapamil in spite of its effect on cardiac and smooth muscle (1,9), causes contraction in chicken biventer-cervicis muscle. This is in consistence with the work of Bondi (10) who demonstrated that verapamil produced calcium release and contracture in frog sartorius muscle. Bondi (10)

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has suggested that the calcium fraction which is released upon addition of verapamil is distinct from the fraction which may be released by caffeine. However, Bondi (10) has reported that verapamil could depress the twitch contraction of frog sartorius muscle, while it had no effect on the twitch contractions of the chicken muscle observed in our experiment (Fig. 2).

Chicken biventer-cervicis muscle contains both slow and fast fibers. At this point it can not be categorically stated whether verapamil causes contraction in both fibers or only in the slow ones.

Glycerol-treated muscles are unresponsive to potassium but sensitive to caffeine indicating selective destruction of the T-tubules (7). The fact that glycerol treatment and omitting calcium from extracellular medium had little effect on contractures caused by verapamil, indicates that T-tubules and extracellular calcium are not involved in verapamil-induced contractions, and possibly verapamil releases calcium from intracellular stores such as sarcoplasmic reticulum. However, when intracellular stores were depleted of their calcium by incubation of muscles in a calcium-free krebs solution in the presence of EGTA, the response of the muscles to verapamil as well as that of caffeine and other drugs was reduced to nil. The ED50 of this effect of verapamil is almost 1000 times higher than its ED50 for suppression of potassium induced contractures of pig coronary strips (1). Therefore, the possibility that this effect of verapamil might have any clinical significance is not very great. The contrary is true for verapamil which is a powerful calcium antagonist in cardiac and smooth muscle (1,9), but actually releases calcium from intracellular stores of skeletal muscles and produces contractures (10 and present work).

These data indicate the possibility of existence of two pharmacologically different calcium channels in respect to calcium antagonists in skeletal and smooth muscle.

1. Fleckenstein A. Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. *A. Rev. Pharmac. Tox.* 17: 149-166; 1977.
2. Hagiwara S. and Byerly L. Calcium channel. *An. Rev. Neurosci.* 4: 69-125; 1981.
3. Rios E. and Brum G. Involvement of dihydropyridine receptors in excitation- contraction coupling in skeletal muscle. *Nature.* 325: 717-720; 1987.
4. Anderson K. and Meissner G. T-tubule depolarization induced SR calcium release is controlled by dihydropyridine receptor and calcium dependent mechanisms in cell hemogenates from rabbit skeletal muscle. *J. Gen. Physiol.* 105: 363-383; 1995
5. Ginsborg BL. and warriner J. The isolated chick biventer-cervicis nerve-muscle preparation. *Br. J. Pharmacol.* 15: 410-411; 1960.
6. Eisenberg B. and Eisenberg RS. Selective disruption of the sacrotubular system in frog sartorius muscle. *J. Cell Biol.* 39: 451-467; 1968.
7. Hall MT., Maleque MA. and Wadsworth RM. The effect of glycerol detubulation, of manganese ions, of dantrolene and of nitrate ions on the responses of isolated chronically denervated soleus muscles of the mouse to acetylcholine. *Br. J. Pharmacol.* 62: 107-113; 1978.
8. Dehpour AR. Mousavizadeh K. and Gerayesh-Nejad S. Calcium release by diltiazem from isolated sarcoplasmic reticulum of rabbit skeletal muscle. *Gen. Pharmacol.* 31: 463-468; 1998.
9. Nabata H. Effects of calcium antagonistic coronary vasodilators on myocardial contractility and membrane potentials. *Japan J. Pharmacol.* 27: 239-249; 1977.
10. Bondi AY. Effects of verapamil on excitation contraction coupling in frog sartorius muscle. *J. Pharmacol. Exp. Ther.* 205: 49-57; 1978.