Effects of Calcium and Magnesium Ions on the Frequency of Miniature End-Plate Potential Discharges in Amphibian Muscle in the Presence of Ethyl Alcohol

In mammalian preparations calcium and magnesium ions influence the frequency of miniature end-plate potentials (m.e.p.p.s)[1], but in amphibian preparations no consistent effects have been detected[2]. On the other hand, in both amphibian and mammalian muscles the amplitude of the end-plate potential is approximately proportional to the external calcium concentration and magnesium acts as an antagonist to calcium.

HUBBARD[1] has suggested that this discrepancy arises because the m.e.p.p.s are of 2 types, one being calcium-independent and unrelated to the end-plate potential, the other calcium-dependent and constituting the component units of the end-plate potential.

In the following experiments, it will be shown that under certain conditions the m.e.p.p.-frequency in frog or toad muscles is approximately proportional to the external calcium concentration within limits and that magnesium acts as an antagonist to calcium.

It has been shown that the m.e.p.p. frequency in rat diaphragm is increased in alcohol[3], and that the frequency in frog sartorius muscles is about 3 times normal in 1% ethanol and is about 7 times normal in 2% ethanol[4]. The increased frequency of m.e.p.p.s in ethanol was constant for a long time after the addition of the drug to the bathing fluid. It is likely that the increase of the frequency is not due to the increase of osmotic pressure by alcohol[5].

The present experiment shows that the m.e.p.p. frequency of frog or toad sartorius muscle fibres was altered in ethanol-Ringer's solution when calcium and magnesium were added to the bathing fluid. In this experiment 90 m*M* calcium chloride solution or 90 m*M* magnesium chloride solution (approximately isotonic solutions) were added to the bathing ethanol-Ringer's solution (10 ml). When 0.1 ml of 90 m*M* $CaCl_2$ solution was added, the concentration of additional calcium was about 0.9 m*M*. Thus the osmotic pressure of the bathing fluid after the addition of calcium or magnesium was not greatly altered, and equivalent changes in the concentration of other ions in Ringer's solution produced little or no effect on m.e.p.p. frequency. The number of m.e.p.p.s recorded intracellularly over a 6–10 min period was counted. The frequencies determined under these conditions were used as control. The frequency of the control (in 2% or 3% ethanol-Ringer's solution) was in the range of 0.26–1.70/sec in toad and 0.41–31.20/sec in frog in the present experiments.

The frequency of m.e.p.p. occurrence was increased when the concentration of the calcium ions was increased from its initial level of 1.8 m*M* in ethanol-Ringer's solution. On the other hand, it was decreased when magnesium was added to the bathing fluid.

When the frequency of m.e.p.p.s was increased after the addition of calcium to the bathing fluid, it was decreased by the further addition of magnesium. And when the frequency of m.e.p.p.s was decreased after the addition of magnesium to the bathing fluid, it was increased by the further addition of calcium. The degree of increase or decrease of the frequency was approximately the same in both toad and frog muscles.

The relation between the frequency of m.e.p.p.s and the concentrations of calcium and magnesium added to the ethanol-Ringer's solution are shown in Figures 1 and 2.

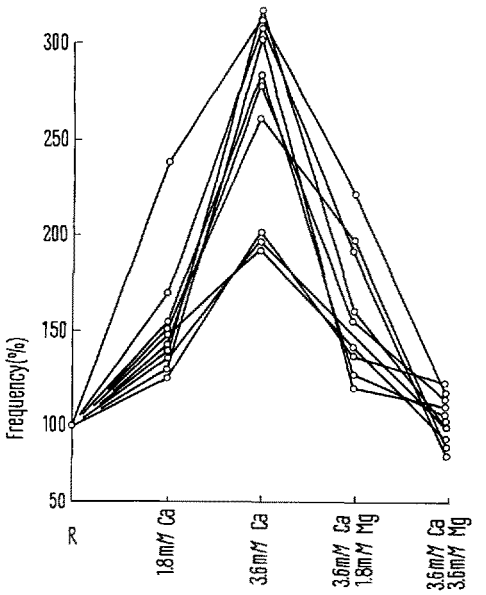

Fig. 1. Relation between the frequency of m.e.p.p.s and the concentration of calcium and magnesium. 2% ethanol was presented throughout the experiments. At first, calcium was added to the bathing fluid and then magnesium. Ordinate: frequency of m.e.p.p.s as % of control. Abscissa: concentration of calcium and magnesium added to the bathing fluid. R: Ringer's solution (control). Each folded line represents a different experiment.

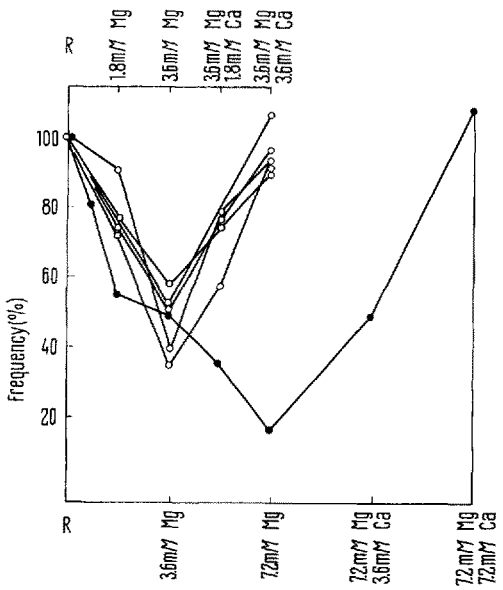

Fig. 2. Relation between the frequency of m.e.p.p.s and the concentration of calcium and magnesium. 3% ethanol was presented throughout the experiments. At first magnesium was added to the bathing fluid and then calcium. Ordinate: frequency of m.e.p.p.s as % of control. Abscissa: concentration of calcium and magnesium added to the bathing fluid. R: Ringer's solution (control). The concentration indicated in the upper part is shown by open circles and that in the lower part by closed circles.

[1] J. I. HUBBARD, J. Physiol., Lond. *159*, 507 (1961).
[2] P. FATT and B. KATZ, J. Physiol., Lond. *117*, 109 (1952).
[3] P. W. GAGE, J. Pharmac. exp. Ther. *150*, 236 (1965).
[4] K. OKADA, in press.
[5] E. J. FURSHPAN, J. Physiol., Lond. *134*, 689 (1956).

When 3.6 mM calcium was added to 2% ethanol-Ringer's solution the frequency of m.e.p.p.s was about 2.5 times control, and was restored approximately to its original value after the further addition of 3.6 mM magnesium to the fluid (Figure 1). When the magnesium concentration was first increased the m.e.p.p. frequency was decreased. At a concentration of magnesium of 7.2 mM in the bathing 3% ethanol-Ringer's solution, the frequency of m.e.p.p.s was about 20% control and was restored to its approximate original value after the further addition of 7.2 mM calcium to the fluid (Figure 2).

It seems likely from these results that calcium and magnesium compete on a one-to-one basis in the mechanism of acetylcholine release from the nerve ending. But the one-to-one basis is formed only on these additional calcium and magnesium ions because Ringer's solution contains 1.8 mM calcium. The mechanism of antagonism of calcium and magnesium on the site related to acetylcholine release might be very complicated at the molecular level.

The results show clearly that calcium ions do have an effect on the frequency of m.e.p.p.s in amphibia just as in mammals, and that magnesium ions act as an antagonist to calcium ions in ethanol-Ringer's solution.

The reason why calcium and magnesium ions are effective on the frequency of m.e.p.p.s in ethanol but not in its absence is not known. It has been suggested that acetylcholine is liberated at the nerve terminal after calcium ions penetrate the cell terminal membrane.

When alcohol is added to the bathing fluid, the permeability of the nerve terminal might be increased. As a result, the frequency of m.e.p.p.s would be increased because some calcium ions which could not penetrate the nerve terminal membrane in normal Ringer's solution are able to do so in the presence of alcohol and thus acetylcholine would be relased from the terminal. The permeability to magnesium ions should also be increased in alcohol. Magnesium ions could penetrate the membrane to compete with calcium ions on the site related to acetylcholine. As a result, spontaneous activity is accelerated by the addition of calcium and depressed by the addition of magnesium[6].

Zusammenfassung. Während bekanntlich Kalzium- und Magnesiumionen keinen Einfluss auf die Frequenz der Spontanrhythmik (m.e.p.p.) der Kaltblütermuskeln in der normalen Ringer-Lösung haben, konnte experimentell festgestellt werden, dass die Frequenz der Spontanrhythmik in einer Äthanol-Ringer-Lösung durch Zusatz von Kalziumionen vermehrt, von Magnesiumionen vermindert wird.

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Le chondriome des hépatocytes en culture trypsinée au cours de la néoglucogénèse. Comportement de la glucose-6-phosphatase

Dans une précédente étude, nous avons montré (HÉBERT et MARCHI[1]), que la forme des chondriosomes dans les hépatocytes en culture trypsinée variait suivant la richesse nutritive du milieu. Normalement, dans les cultures bien nourries, le chondriome se présente sous la forme granuleuse. NOËL[2] a montré que cet aspect, dans le lobule hépatique, s'observe au niveau de la zone périphérique et correspond à un état de fonctionnement actif. Dans les cultures plus âgées, dont le milieu s'appauvrit, les mitochondries se transforment progressivement en batonnets, image que NOËL a décrite dans la zone centrale du lobule et qui correspond à un état de repos.

Nous avons pu provoquer la transformation des mitochondries granuleuses en batonnets en diluant de moitié le milieu de culture habituel avec de la solution de Ringer. Le processus est rapide. On peut l'observer 5 h après que les cultures aient été placées dans le milieu dilué. L'appauvrissement expérimental du milieu de culture met les hépatocytes en état de fonctionnement ralenti et provoque la transformation de leur chondriome.

Nous nous sommes demandé si, en maintenant les hépatocytes en activité métabolique, nous pourrions empêcher cette transformation de se produire. Nous avons observé[3] que l'addition au milieu de culture d'acides aminés glyco-formateurs, tels que le glycocolle et la sérine, provoquait dans les hépatocytes en culture, un processus de néoglucogénèse. La charge en glycogène des hépatocytes augmente et peut se maintenir plusieurs jours, tandis que la teneur en glucose du milieu de culture augmente. Les hépatocytes sont ainsi maintenus en état de fonctionnement actif: d'une part en raison du processus biochimique qui caractérise la néoglucogénèse, en particulier le processus de désamination des acides aminés, d'autre part en raison de l'enrichissement du milieu en glucose.

Nous avons donc porté notre étude sur le chondriome d'hépatocytes cultivés dans un milieu additionné de glycocolle. Il s'agit toujours de cultures trypsinées de foie d'embryon de poulet de 8–9 jours réalisées en tubes de Leighton-Barski. Aux 2 ml de milieu de culture (Milieu 199 de l'Institut Pasteur et sérum de poulain) on ajoute 1 ou 2 gouttes d'une solution de 100 mg de glycocolle pour 5 ml de sérum physiologique. Les cultures sont examinées au bout de 24, 48 et 72 h. Après fixation adéquate, elles sont traitées par la réaction de l'acide périodique Schiff pour la mise en évidence du glycogène (avec contrôle par l'amylase), par la technique classique d'Altmann pour le chondriome. Enfin, nous avons procédé à la mise en évidence de la glucose-6-phosphatase par la réaction de Chiquoine.

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[2] R. NOËL, *Recherches histo-physiologiques sur la cellule hépatique des mammifères*, Thèse Sciences, Paris (1922).
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