[13] *Lutsch, G., Venker, P.:* Intracytoplasmatische Membranen in E.coli nach Magnesium- bzw. Phosphatmangel. Naturwissensch. **56** (1969) 568.
[14] *De Mendonca, M., Grichois, M.-L., Garay, P., Sassard, J., Ben-Ishay, D., Meyer, P.:* Abnormal net Na^+ and K^+ fluxes in erythrocytes of three variations of genetically hypertensive rats. Proc. Nat. Acad. Sci. **77** (1980) 4283—4286.
[15] *Morgan, C., Rosenkranz, H. S., Rose, H. M.:* Electron microscopy of magnesium-depleted bacteria. J. Bacteriol. **91** (1966) 891—895.
[16] *Rinaldi, M. L., Le Peuch, C. J., Demaille, J. G.:* The epinephrine-induced activation of the cardial slow Ca^{2+} channel is mediated by the cAMP-dependent phosphorylation of calciductin, a 23 000 Mr sarcolemmal protein. FEBS Letters **129** (1981) 277—281.
[17] *Sachs, J. B.:* Ouabain-insensitive sodium movements in the human red blood cell. J.gen. Physiol. **57** (1971) 259—282.
[18] *Sarkadi, B., Tosteson, D.C.:* Active cation transport in human red cells. In: Giebisch, G., Tosteson, D.C., Ussing, H.H., [eds.] Membrane Transport in Biology, Vol. II, Springer-Verlag, Berlin, Heidelberg, New York, (1979) 116—160.
[19] *Schilling, K., Börnig, H., Cumme, G., Hoppe, H.:* Effect of magnesium-dependent cell membrane alterations on the transport of K^+ in Ehrlich ascites tumor cells. Acta biol. med. germ. **39** (1980) 177—184.
[20] *Vormann, J., Günther, T., Leder, O.:* Effects of isoproterenol and phenylephrine on the hearts of Mg-deficient rats. Magnesium-Bull. **3** (1981) 130.
[21] *Whang, R., Welt, L.G.:* Observations in experimental magnesium depletion. J. Clin. Invest. **42** (1963) 305—313.
[22] *Wiley, J.S., Cooper, R. A.:* A furosemide-sensitive cotransport of sodium plus potassium in the human red cell. J. Clin. Invest. **53** (1974) 745—755.

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Reduction of extracellular Mg^{2+} and Ca^{2+} concentration by anesthetics*)

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Zusammenfassung

Die Mg^{2+} Konzentration im Serum wurde bei Ratten durch Äther um 2 %, durch Nembutal (Pentobarbital) (60 mg/kg) um 3 %, durch Inactin (Thiobutabarbital)(100 mg/kg) um 7 % und durch Halothan um 12 % gesenkt. Die $[Ca^{2+}]$ wurden in ähnlicher Weise gesenkt. Die $[Na^+]$ und $[K^+]$ wurden in vivo nicht signifikant verändert.

Die Inactin-Wirkung wurde an Rattenerythrocyten in vitro analysiert. Der Abfall der $[Mg^{2+}]$ und $[Ca^{2+}]$ erfolgt schnell und stellt einen neuen steady state abhängig von der Inactinkonzentration dar. Der Abfall der $[Mg^{2+}]$ und $[Ca^{2+}]$ erfolgt unabhängig voneinander und verschwindet bei niedriger extrazellulärer $[Mg^{2+}]$ oder $[Ca^{2+}]$. Bei hoher Inactinkonzentration treten K^+ und Hämoglobin aus den Erythrozyten aus.

Die Ergebnisse lassen sich mit einer erhöhten Membranpermeabilität erklären.

Summary

Serum Mg^{2+} concentration in rats was reduced in vivo by ether by 2 %, by Nembutal (Pentobarbital) (60 mg/kg) by 3 %, by Inactin (Thiobutabarbital) (100 mg/kg) by 7 % and by halothane (1.5 %) by 12 %. $[Ca^{2+}]$ in serum was similarly reduced. $[Na^+]$ and $[K^+]$ were not significantly affected in vivo.

The effect of Inactin was analyzed in vitro with rat erythrocytes. The decrease in $[Mg^{2+}]$ and $[Ca^{2+}]$ occurs rapidly, is constant with time, establishing a new steady state and increases with Inactin concentration. The decrease in $[Mg^{2+}]$ and $[Ca^{2+}]$ is independent of each other and disappears at low extracellular $[Mg^{2+}]$ or $[Ca^{2+}]$. At high Inactin concentration K^+ and hemoglobin leak out of the erythrocytes.

The results can be explained by an increased membrane permeability.

*) Results presented at the 3rd International Symposium on Magnesium, Baden-Baden, 22. 28. 8. 1981.

Resumé

Les concentrations sériques de Mg^{++} ont été réduites de 2 % in vivo par l'éther, de 3 % par le nembutal (pentobarbital) (60 mg/kg), de 7 % par l'Inactine (thiobutobarbital) (100 mg/kg) et de 12 % par l'halothane (1,5 %). Le $[Ca^{++}]$ sérique a été réduit de façon similaire. $[Na^+]$ et $[K^+]$ n'ont pas été affectés de façon significative in vivo.

L'effet de l'Inactine a été analysé in vitro avec des érythrocytes de rat. La réduction de $[Mg^{++}]$ et de $[Ca^{++}]$ se produit rapidement, elle est constante avec le temps en établissant un nouvel état constant et elle s'est accrue avec la concentration d'Inactine. Les réductions de $[Mg^{++}]$ et de $[Ca^{++}]$ sont indépendantes l'une de l'autre et elles disparaissent pour de faibles concentrations extracellulaires de $[Mg^{++}]$ ou de $[Ca^{++}]$. Pour une concentration élevée d'Inactine, du K^+ et de l'hémoglobine ont été libérés des érythocytes.

Les résultats peuvent être expliquer par une perméabilité accrue des membranes.

* * *

During our experiments on the Mg metabolism in rats we found reduced Mg^{2+} concentration in the serum of rats anesthetized with Inactin or halothane. Therefore, we investigated this effect in detail.

Methods

From male Wistar rats weighing about 300 g, blood was taken with capillaries after cutting the tip of the tail.

Mg^{2+} and Ca^{2+} concentrations in the serum or incubation medium were measured by atomic absorption spectrometry. For in vitro incubations heparinized blood was taken from the heart under ether anesthesia. Blood was taken directly or washed and resuspended in the same volume of incubation medium, containing (in mmol/l) 150 NaCl, 5 KCl, 3 Na phosphate, 3 Tris (pH 7.4), 5 glucose, $MgCl_2$, and $CaCl_2$ as indicated. The incubations were performed in Warburg vessels at 37° C. At time zero and at various times after the addition of Inactin, 0.5 ml samples were centrifuged in an Eppendorf Microcentrifuge for 1 min, 0.1 ml of the supernatants were taken for the determination of Mg^{2+}, Ca^{2+} or glucose concentration. Glucose was determined enzymatically (Enzymatischer Farbtest, Nr. 166 391, Boehringer, Mannheim).

Results

The effect of various anesthetics on the Mg^{2+} and Ca^{2+} concentrations in rat serum is shown in Tab. 1. The degree of (Mg^{2+}) and (Ca^{2+}) reduction followed the pattern: ether < Nembutal < Inactin < halothane. (Mg^{2+}) and (Ca^{2+}) were affected to about the same degree.

As was demonstrated with Inactin (Fig. 1), the reduction of (Mg^{2+}) and (Ca^{2+}) occurred rapidly and thereafter was constant during the experiment for up to 60 min. Under these conditions, there seemed to be no self-regulation of (Ca^{2+}) and (Mg^{2+}) e.g. by PTH. The reason for this behaviour remains open.

The reduction of extracellular (Mg^{2+}) and (Ca^{2+}) by Inactin could also be observed with isolated rat erythrocytes in vitro (Fig. 2). Again, there was a rapid reduction of (Mg^{2+}) and (Ca^{2+}), depending on the Inactin concentration. The reduction of (Mg^{2+}) was independent of (Ca^{2+}) and vice versa (Fig. 3). Therefore, it can be concluded that there was no coupling between Mg^{2+} and Ca^{2+}. At low extracellular concentration, there was no reduction of (Mg^{2+}) or (Ca^{2+}) indicating a passive process.

Specifity

The in vivo effect of Inactin was specific to (Mg^{2+}) and (Ca^{2+}). Both the Na^+ and K^+ concentrations in serum were not significantly altered. However, in vitro, at high Inactin conentrations (6 mg/ml), K^+ and hemoglobin leaked out of the erythrocytes.

Tab. 1: Mg^{2+} and Ca^{2+} concentration in rat serum before and 20 min after anesthesia. Mean ± SEM of 4 rats.

		$[Mg^{2+}]$ mmol/l		$[Ca^{2+}]$ mmol/l	
		0	20 min	0	20 min
Ether		1.00 ± 0.02	0.98 ± 0.02	2.52 ± 0.06	2.45 ± 0.04
Nembutal	60 mg/kg i.p.	0.97 ± 0.02	0.94 ± 0.02	2.53 ± 0.04	2.45 ± 0.05
Inactin	100 mg/kg i.p.	0.97 ± 0.02	0.90 ± 0.01	2.54 ± 0.05	2.40 ± 0.03
Halothane	1.5 %	0.97 ± 0.02	0.85 ± 0.03	2.55 ± 0.04	2.30 ± 0.06

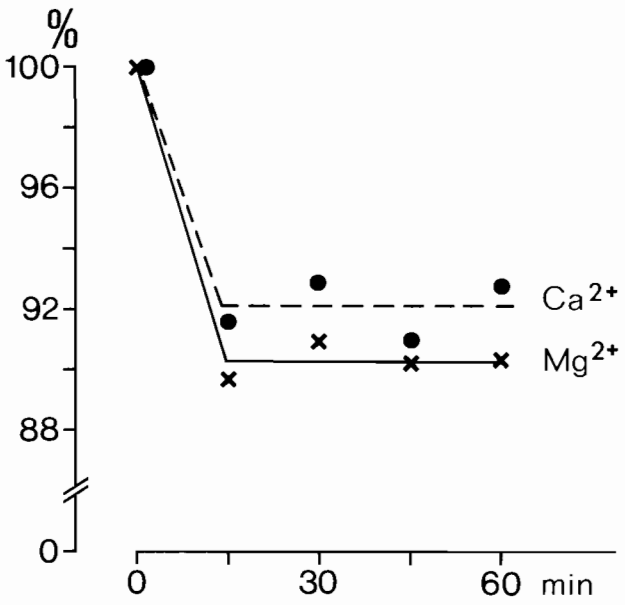

Fig. 1: Reduction of Mg^{2+} and Ca^{2+} concentration in the serum of rats after injection of Inactin [100 mg/kg i.p.]. Blood was taken from the tail at times indicated. Ca^{2+} [●], Mg^{2+} [X].
Mean of 4 rats.

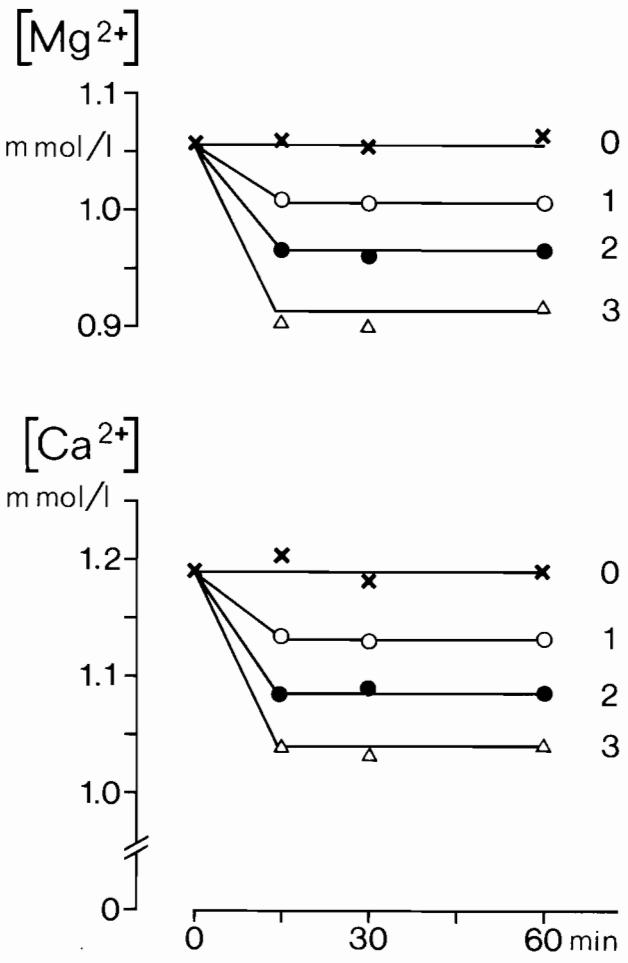

Fig. 2 Reduction of extracellular Mg^{2+} and Ca^{2+} concentration in erythrocyte suspensions. Erythrocytes were incubated in saline with 1.05 mmol/l $MgCl_2$ and 1.19 mmol/l $CaCl_2$. At time zero Inactin in saline was added. Inactin concentrations: 1, 0.6 mg/ml; 2, 1.8 mg/ml; 3, 6 mg/ml; 0, without Inactin.
Mean of 3 experiments in duplicates.

Metabolic effects

After incubation in vitro we found no significant increase of glycolysis in rat erythrocytes due to Inactin.

Discussion

Anesthetics are known to react with phospholipids and/or proteins of membranes. For literature see [3]. The quantitatively different effect of various anesthetics may be caused by their different oil/water partition coefficient or different binding (solubility) to the membrane. The reduction of extracellular (Mg^{2+}) and (Ca^{2+}) by the anesthetics may be produced by an increased passive cell membrane permeability and intracellular uptake of Mg^{2+} and Ca^{2+}.

The following mechanism can be suggested. Cardiolipin may function as a ionophore for Ca^{2+} and Mg^{2+} [2]. The association of cardiolipin with an anesthetic may lead to the formation of a complex with increased translational mobility of the phospholipid in the membrane phase and thus an increased translocation of Ca^{2+} and Mg^{2+} across the membrane [2]. The resulting steady state may be caused by an increased transport of Mg^{2+} and Ca^{2+} out of the cells. The situation may be similar for Na^+ and K^+. As the capacity of the Na^+-K^+ pump is higher, the leak of K^+ owing to low Inactin concentrations may be compensated by an increased transport rate. Alternatively, Na^+ and K^+ may not be involved in the permeability increase caused by the anesthetics because of their lower affinity to phospholipids. In this case the insignificantly increased rate of glycolysis in the experiments with erythrocytes may be explained by a low capacity of their Ca^{2+} ATPase. However, a secondarily increased pump rate is not established. Therefore, an increased binding of Mg^{2+} and Ca^{2+} in the presence of anesthetics cannot be excluded by the results of our experiments. Further clarification is required by means of isotopic flux measurements.

The participation of other cells was not studied. With the analogous barbiturate Thiopental an inhibition of influx and efflux of $^{42}K^+$ and $^{45}Ca^{2+}$

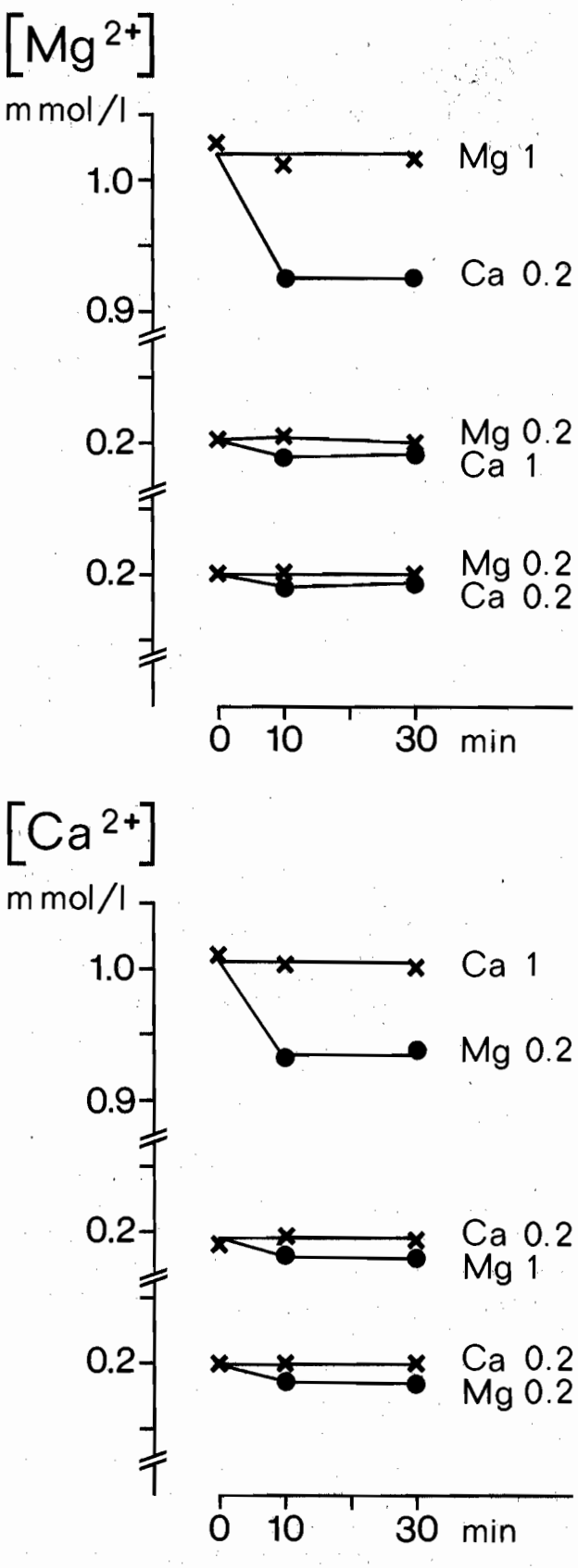

Fig. 3: Effect of Inactin [1.5 mg/ml] on extracellular Mg^{2+} and Ca^{2+} concentration in erythrocyte suspensions. Erythrocytes were incubated in saline solution with $[Mg^{2+}]$ and $[Ca^{2+}]$ as indicated.
Mean of 2 experiments in duplicates.

in the myocardium was found to occur [1]. There may be differences in the anesthetics-induced uptake of Mg^{2+} and Ca^{2+} by various cells because the process may depend on membrane potential and concentration gradient.

References:

[1] *Frankl, W. S., Poole-Wilson, P. A.:* Effects of Thiopental on Tension Development, Action Potential and Exchange of Calcium and Potassium in Rabbit Ventricular Myocardium. J. Cardiovasc. Pharmocol. **7** (1981) 554—565.

[2] *Mies, R., Hamacher, J.:* Tierexperimentelle und klinische Untersuchungen der inotropen Wirkung von Kalium-Magnesium-Asparaginat unter Halothannarkose Belastung. Naunyn-Schmiedebergs Arch. Pharmocol. **251** (1965) 149—150.

[3] *Tyson, C. A., Zande, H. V., Green, D. E.:* Phospholipids as Ionophores. J. Biol. Chem. **251** (1976) 1326—1332.

[4] *Vanderkooi, G., Shaw, J., Storms C., Vennerstrom R., Chignell, D.:* On the mechanism of action of anesthetics. Direct inhibition of mitochondrial F_1-ATPase by n-butanol and tetracaine. Biochim. Biophys. Acta **635** (1981) 200—203.

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