Hepatic cyclic AMP levels in rats after injection of saline, lead, endotoxin, and glucagon[a]

Treatment	Time after injection			
	1 min	5 min	15 min	4 h
Sodium acetate + saline (control)	0.57 ± 0.1 (5)	0.63 ± 0.1 (3)	0.83 ± 0.1 (3)	0.82 ± 0.1 (3)
Sodium acetate + endotoxin (3.3 mg/0.5 cm³)	0.61 ± 0.1 (5)	0.77 ± 0.1 (5)	0.64 ± 0.1 (5)	1.1 ± 0.02 (2)
Lead acetate + saline	0.52 ± 0.1 (3)	0.57 ± 0.1 (3)	0.71 ± 0.1 (3)	0.82 ± 0.2 (2)
Lead acetate + endotoxin (0.5 µg/0.5 cm³)	0.51 ± 0.1 (4)	0.79 ± 0.1 (4)	0.81 ± 0.2 (4)	0.95 ± 0.4 (2)
Sodium acetate + glucagon (1 mg/0.5 cm³)	7.2 ± 1.3[b] (2)	4.6 ± 0.5[b] (2)	4.4 ± 1.8 (2)	1.3 ± 0.1 (2)

[a] Data is expressed as picomoles cAMP/mg wet weight of liver. Values represent the mean ± SEM, and the number in parentheses indicates the number of animals per group. [b] $p < 0.01$ vs. control.

Materials and methods. After Nembutal® anesthesia, male Sprague-Dawley rats (170–220 g) were given i.v. injections (0.5 ml) of the following: sodium acetate (4.3 mg); lead acetate (20 mg); endotoxin (3.3 mg or 0.5 µg); phosphate-buffered saline; or glucagon. *Serratia marcescens* endotoxin (Lot No. 582087), prepared by the BOIVIN method, was obtained from Difco Laboratories (Detroit, Michigan). At various time intervals post-injection, rats were killed by submersion in liquid nitrogen. The frozen liver was isolated, extracted, and analyzed for cAMP, using the competitive binding method of GILMAN[7].

Results and discussion. The results, summarized in the Table, show that control injections of sodium acetate and saline caused no change in hepatic cAMP. Glucagon was used as a positive control, and, as expected, produced significant elevations in hepatic cAMP in the 1- and 5-min time intervals, post-injection. However, under the same conditions, rats given a LD_{50} dose (3.3 mg) of *S. marcescens* endotoxin showed no change in cAMP concentrations. Liver samples were examined as much as 4 h post-injection, when some of the endotoxin-treated animals had begun to die. Even at that time, there was no significant alteration in cAMP levels, indicating that control of hepatic cAMP may not be a significant step in the mechanism of endotoxin toxicity. These results, however, do not rule out the possibility of very small, localized changes in hepatic cAMP concentrations, which affect carbohydrate metabolism, but are not detected in measurements of the total hepatic cAMP pool. In contrast, bacterial exotoxins, such as cholera toxin, have been shown to markedly elevate cAMP in the intestinal mucosa[8].

An additional group of rats was treated with lead acetate at a concentration which markedly sensitizes these animals to very small quantities of endotoxin[5,6]. Lead acetate is known to inhibit a number of enzymes, including cyclic nucleotide phosphodiesterase[9], and since this enzyme is important in cAMP metabolism, this would be a reasonable mechanism to explain the lead-endotoxin interaction. In the present study, however, when lead was given alone, or in combination with LD_{50} endotoxin, there was no significant change in endogenous cAMP (Table). Thus, the mechanism of lead acetate sensitization is not by means of marked elevations in hepatic cAMP, and alternative explanations should be considered for the meachnism of endotoxin lethality and lead-sensitization.

Zusammenfassung. Nachweis, dass die Konzentration von zyklischem AMP in Lebern normaler und Blei-sensibilisierter Ratten durch i.v. Injektionen von *Serratia marcescens* Endotoxin nicht beeinflusst wird.

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Influence of Calcium on the Interaction of Anesthetic Drugs with Artificial Phospholipid Membranes

In a previous paper[1], we reported a study on the effect of 2 drugs with local anesthetic properties (tetracaine and hexobarbital) on the electrical resistance of artificial phospholipid membranes (bilayers). Both drugs increase the resistance probably by forming hydrophobic bonds, but ionic interactions may alter drug activity by modifying the local concentration at the lipid-water interface.

It has been proposed[2,3] that calcium and local anesthetics compete with one another with respect to their

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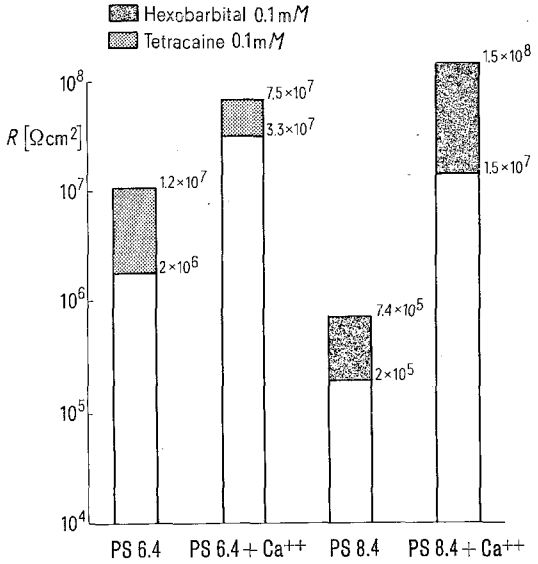
The effect of calcium on the PE and PS bilayer membranes expressed by the ratio between the resistance produced by 0.1 m*M* $CaCl_2$ and the intrinsic bilayer resistance (R_o).

	PE 6.4	PS 6.4	PS 8.4
$R_o(\Omega \cdot cm^2)$	4×10^5	2×10^6	2×10^5
$R_{Ca}(\Omega \cdot cm^2)$	4.2×10^5	3.3×10^7	1.5×10^7
R_{Ca}/R_o	1.05	16.50	75

actions on the nerve membrane, and that this antagonism may be the basis of the local anesthesia. The negative groups of phospholipid molecules might serve as the binding site for calcium and local anesthetics on membranes. Previous work with model systems produced evidence on calcium-local anesthetic antagonism[4,5].

In the present study, the influence of calcium on the increase of resistance produced in artificial phospholipid membranes by tetracaine and hexobarbital is analyzed. Since we suspect ionic interactions between calcium and the anesthetic drugs, we use them under conditions in which they are highly dissociated (pH 6.4 for tetracaine and pH 8.4 for hexobarbital).

The membranes were formed with *n*-decane solution of phosphatidylethanolamine (PE) or phosphatidylserine (PS). The electrical resistance was determined as previously described[1]. The anesthetic drugs and/or $CaCl_2$ were always added to the 2 cell halves before forming the membranes. The aqueous solution contained 100 m*M* KCl buffered at pH 6.4 with 5 m*M* *Tris*-maleate or at pH 8.4 with 5 m*M* *Tris*-HCl.



The effect of 0.1 m*M* tetracaine and hexobarbital on the resistance of phosphatidylserine bilayer membranes formed before and after the addition of 0.1 m*M* $CaCl_2$ to a 100 m*M* KCl solution buffered at pH 6.4 or 8.4.

The presence of calcium in the bathing solution (0.1 m*M* $CaCl_2$) greatly affected the electrical resistance of the membranes and their response to the anesthetic drugs. While calcium had no significant effect on the resistance of a neutral bilayer (PE at pH 6.4), it increased more than 15 times (PS at pH 6.4) and 75 times (PS at pH 8.4) the resistance of negatively charged membranes as reported in the Table.

The increase of the PS membrane resistance due to the cationic tetracaine at 0.1 m*M* and pH 6.4 was reduced $2^1/_2$-fold by the addition of 0.1 m*M* $CaCl_2$. In contrast, calcium enhanced by about the same amount the effect of the anionic hexobarbital on the resistance of the PS membrane at pH 8.4 (Figure). The effect of calcium on the increase of resistance produced by anesthetic drugs can be simply[1] explained according to the model outlined previously. It is known that calcium binds to the carboxyl group of the PS membranes, forming a di-soap with 2 adjacent phospholipids[6]. The surface charge should be partially neutralized (from 2 to 1 negative charge per molecule) and consequently the negative surface potential reduced. Since calcium influences the action of tetracaine and hexobarbital on the PS membrane in a opposite way – i.e. it inhibits tetracaine and potentiates hexobarbital – it may be argued that again a reduction of the surface potential leads to different interfacial and consequently intramembrane concentration for the two anesthetics.

It is clear from the above discussion that the interaction of calcium and anesthetic drugs with the permeability properties of the phospholipid bilayer can be interpreted as indicating different sites of action. Further support for this suggestion comes from the fact that calcium and drugs with local anesthetic properties increase the bilayer resistance by different mechanisms. 1. Calcium does not modify the resistance of the neutral bilayers (PE at pH 6.4) which are, in contrast, strongly affected by the anesthetics. 2. The effect of a solution containing both calcium and hexobarbital on the PS membrane is greater that the sum of the separate effects of the two agents. If the site of action were the same, we should expect competition and consequently a reduced effect. It is interesting to note that recently ARHEM and FRANKENHÄUSER[7] reinvestigated the effects of local anesthetics and calcium on permeability properties of nodal membrane in myelinated nerve fibres and showed consistently that the effect of local anesthetics and of changes in calcium concentration were independent of each other.

Riassunto. Viene valutata l'influenza dello ione calcio sulla interazione tra due sostanze ad azione anestetica locale (esobarbital e tetracaina) e membrane fosfolipidiche artificiali (bilayers) con diversa carica superficiale. I risultati indicano che il calcio e gli anestetici interagiscono con un diverso meccanismo con la molecola fosfolipidica e in particolare non competono per un medesimo sito d'azione.

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