Extracellular Calcium Concentration and Catecholamine Release from Adrenal Medulla by Acetylcholine and Potassium

DOUGLAS and POISNER showed that washout of ^{45}Ca from cat adrenals was relatively rapid for about 50 min and that subsequent ^{45}Ca washout occurred linearly at a slow rate[1]. 'The curves are very similar to those obtained by others in related studies on nerve and muscle in which the rapid phase is held to reflect loss from the extracellular space, and the slow phase loss from the cells'[1]. However, perfusion of cat adrenals with a calcium-free medium for 16 min practically abolishes the response of the adrenal medulla to acetylcholine or potassium[2]. Thus, it appeared that the loss of response to these agonists occurred before washout of extracellular calcium. This study was undertaken to more clearly define the relationship between extracellular calcium concentration and acetylcholine or potassium induced catecholamine release from adrenal medulla.

Methods. Fresh bovine adrenals were perfused through the adrenal vein with aerated Lockes solution, 25°C, at a rate of 5 ml/min as previously described[3]. The perfusion fluid was collected after passing out from the many small arteries of each gland. Agonists were infused for 2 min periods (10 ml total volume). Catecholamines (epinephrine and norepinephrine) were determined colorimetrically in aliquots of the perfusates[4]. Amine release was estimated for the 2-min periods during which the agonists were present.

When calcium concentration was altered in the perfusing medium, sodium concentration was correspondingly altered to maintain isotonicity.

Results. Figure 1 shows that the response of bovine adrenals to potassium and to acethylcoline decrease in parallel to about 7% of control at 6 to 8 min after beginning perfusion with a calcium-free medium.

To estimate the minimal amount of calcium needed in the extracellular fluid to support a response to acetylcholine, the calcium concentration of the Lockes solution was lowered to between 10 and 30% of that in normal Lockes solution. Figure 2 shows that the response to acetylcholine drops to about 10% of control when the extracellular calcium concentration is lowered to 12.5% of normal. Therefore, when the response to acetylcholine declines to about 7% of control, 6 to 8 min after initiation of perfusion with a calcium-free solution as in Figure 1, the calcium concentration in the interstitial fluid is estimated to be 0.26 mM. These experiments show that it is not necessary to remove all the calcium from the extracellular compartment of adrenal medulla in order to decrease acetylcholine-induced catecholamine release to an undetectable level.

Discussion. The responses of bovine adrenal medulla to acetylcholine and potassium are lost in parallel when the glands are perfused with a calcium-free medium. Assuming that catecholamine release is proportional to calcium entry into medullary cells, calcium influxes caused by acetylcholine or potassium appear to be similarly affected by the calcium concentration in the perfusing medium.

Essentially no catecholamine release from adrenal medulla occurred in response to acetylcholine when the

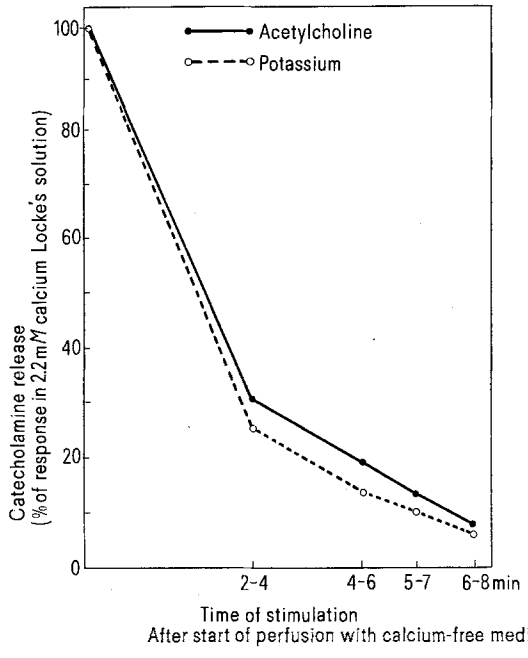

Fig. 1. Loss of response of bovine adrenals to acetylcholine or potassium in calcium-free medium. Glands were stimulated with acetylcholine (100 μg/cm³, 10 cm³) or potassium (56 mM, 10 cm³) at 2, 4, 5 or 6 min after changing from a calcium containing to a calcium-free perfusion medium. Catecholamine release is expressed as a percent of the control responses to these same agonist concentrations obtained about 20 min prior to the response in calcium free medium. Each point is a mean of 9 to 20 determinations except for the 5 to 7 min interval where the mean of 4 determinations is shown. Mean control responses to acetylcholine and potassium were 77 ± 6 and 104 ± 12.7 μg/min ± S.E. above resting secretion.

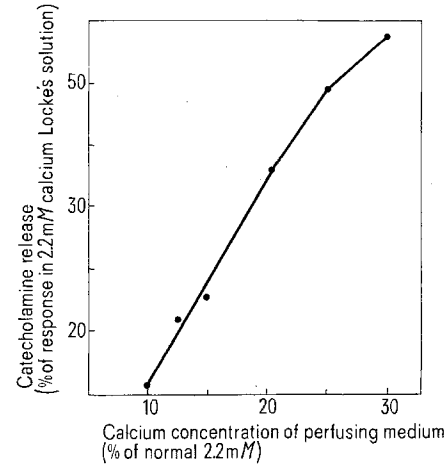

Fig. 2. Effect of calcium concentration of the perfusion medium on the response of bovine adrenals to acetylcholine. Glands were stimulated with acetylcholine (100 μg/cm³, 10 cm³) 20 min after changing from a medium containing 2.2 mM calcium to a low calcium medium. Catecholamine release was measured in aliquots of the perfusates, and is expressed as a percent of control responses to the same amount of acetylcholine obtained in 2.2 mM Lockes solution, 10 min before introduction of low calcium media. (Mean control catecholamine release = 70 μg/min above resting secretion). Each point is a mean of 4 determinations.

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calcium concentration of the perfusing medium was lowered to 0.22 m*M*. Apparently a minimum calcium concentration gradient across the medullary cell membrane must exist before acetylcholine-induced catecholamine release can occur.

Zusammenfassung. Nachweis an der isolierten perfundierten Rindernebenniere (5 ml/min), dass die Acetylcholin- oder Kalium-Stimulation der Catecholaminausschüttung bei fehlendem Calcium gleichmässig verhindert war. Ferner wurde die geringste Calciumkonzentration für den Ausschüttungsreiz bestimmt.

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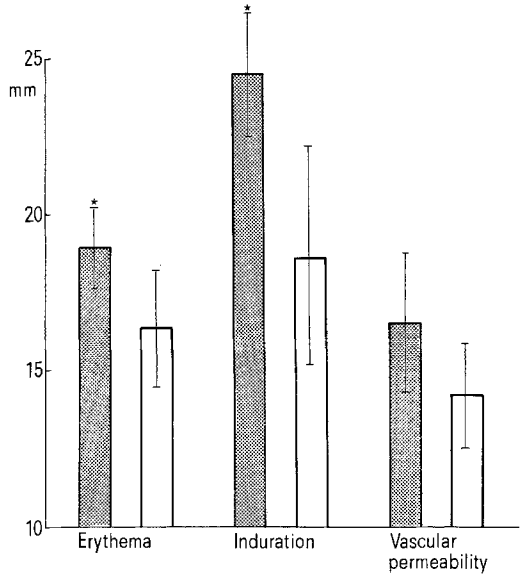
The Effect of N-(2-hydroxyethyl)-Palmitamide on Delayed Hypersensitivity in Guinea-Pig

It has been shown that N-(2-hydroxyethyl)-palmitamide (PEA) can decrease the intensity of several inflammatory and immunological processes in experimental animals[1–3]. Recently the interest on biological properties of PEA has been revived because of its capacity to increase nonspecific tolerance to several bacterial toxins[4]. In this communication the influence of PEA on some manifestation of tuberculine hypersensitivity in guinea pigs is reported.



Erythema, induration and vascular permeability of the skin tests in tuberculine hypersensitivity in guinea pigs. ▦, N-(2-hydroxyethyl)-palmitamide pretreated animals; □, controls. The values of induration are expressed as 10-fold magnification of skin double thickness measured by 'Schnelltaster' System Kröplin. The transverse diameter of erythema was measured with a millimeter ruler. Each group consists of 7 animals (mean ± limits of confidence). * $P < 0.05$ compared to controls.

Material and methods. Random bred male albino guinea-pigs weighing 250–350 g were used. Animals were fed with 50 mg of PEA per kg body wt. and day by stomach tube. The animals received PEA 13 times prior to sensitization, the last dose of PEA was administered on the day of sensitization. The animals were injected with 1 ml of Freund's complete adjuvant per animal into 4 foot pads and the neck. 3 weeks later the skin test, vascular permeability test and inhibition of migration of macrophages were performed. Skin tests were performed on shaven skin of dorsal flank of animals. 10 μg of PPD in 0.1 ml of saline was administered intradermally, while controls were given saline only. The vascular permeability tests were performed by i.v. injection of Evans blue in saline and measurements evaluated after 30 min and 24 h respectively as described previously[5].

The test of inhibition of migration of macrophages was performed on guinea pig spleen explants by a method described elsewhere[6].

Results. We have found that animals treated with PEA prior to sensitization displayed significant increase in the erythema intensity, diameter and induration values 24 h after PPD administration as compared to the controls (Figure). These results were in good accordance with values obtained by measuring vascular permeability. In the 30 min interval, the blueing displayed-as expected-minor values in both control and pretreated animals. On the other hand, in 24 h interval the PEA pretreated animals showed marked increase in diameter as compared to controls (Figure).

When spleen fragments from hypersensitive animals were tested on inhibition of macrophage migration in

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Mean values of cytotoxic indices (CI) from controls and N-(2-hydroxyethyl)-palmitamide pretreated animals

N-(2-hydroxyethyl)-palmitamide pretreated group	Individual animals 0.26 0.23 0.29 0.25 0.24	Mean ± limits of confidence 0.25 ± 0.026
Control group	0.65 0.38 0.25 0.26 0.40	0.39 ± 0.179

Each value of individual animal represents an average plotted as a mean of 9 spleen fragments.