

containing neurons of the brain must cause 5-HT release and thereby induces SWS. This supports the notion that in the brain a loss in the tonic activity of ACh containing neurons leads to 5-HT release which in turn induces SWS.

After PCPA treatment, the cats failed to show deep-SWS and the background electrical activity level during light-SWS was high in low voltage fast activity (Figure C) compared to the electrical activity level before the PCPA treatment (Figure A). This may be explained on the basis that the initiation of light-SWS probably takes place with the release of 5-HT and maintenance of this phase leading to deep-SWS is the consequence of 1. an availability of more 5-HT, and 2. accumulation and escape of its intermediate metabolite from the synaptic cleft to extracellular spaces in adequate concentration. Consistent with this view is the finding that 100 ng of 5-hydroxytryptophol in 50 μ l sterile saline, when injected into the 4th ventricle of cats with a chronic recording device, induces both behavioral and EEG signs of SWS, whereas a dose of 50 ng was not effective in inducing SWS (unpublished observation).

Zusammenfassung. Hemicholinium-3 (HC-3) in den 4. Ventrikel der Katze verabreicht, bewirkt langsamen Schlaf. Vorbehandlung mit *para*-Chlorophenylalanin vermindert die Wirksamkeit des HC-3. Hemmung zentraler cholinergischer Nervenzellen führt vermutlich zur Freisetzung von Serotonin und damit zu langsamem Schlaf.

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Properties of Granules that Contain Kallikrein and Renin¹

The richest source of kallikrein in the body is the submaxillary gland of the rat². A significant amount of the enzyme in this gland is located in subcellular particles, in granules³. The submaxillary gland of the white adult male mouse contains less kallikrein but it has renin as well in large quantities⁴⁻⁷. Granules obtained from the homogenized gland of the mouse released both kallikrein and renin. 4 different centrifugation procedures, however, have failed to separate the kallikrein and renin containing particles⁷. The present report describes some additional properties and separation of kallikrein and renin containing the granules.

Materials and methods. White, over 60-day-old Swiss male mice (Webster) were sacrificed and the submaxillary glands were homogenized as described previously⁷. The isolated granules were collected at 480 g in a 0.25 M sucrose solution or at 800 g in a 0.88 M sucrose solution buffered with 5 mM Tris of pH 7.4. The enzymic activities of the granules were determined after lysing them with 0.1% Triton X-100 or after repeated freezing and thawing^{3,7}. Millipore filtration was carried out in a 0.88 M sucrose solution with a Swinny adapter attached to a 3 ml syringe. The pore size of the filters ranged from 0.45 to 3.0 μ m.

Isopycnic gradient centrifugation was performed in a discontinuous sucrose density gradient ranging from 1.62 M to 1.84 M with an increase in molarity of 0.02 for each layer. The centrifugation was done at room temperature for 4 h at 40,000 g in a Spinco L-2 65 preparative ultracentrifuge.

Rate zonal centrifugation was carried out in a discontinuous sucrose density gradient using Spinco band forming caps. The gradient was formed by layering 6 times 2 ml volume of sucrose solution of molarities increasing stepwise from 1.0 to 1.5 M. A SW 25.1 rotor was used in the ultracentrifuge at room temperature.

The hydrolysis of benzoyl-L-arginine ethyl ester (BAEE) was determined in a Cary recording UV-spectrophotometer⁸. Renin activity was measured by assaying the amount of angiotensin liberated by the enzyme from purified angiotensinogen on the systemic arterial blood pressure of the pithed rat⁷. Kinin release by kallikrein from a

purified human kininogen substrate was followed on the isolated rat uterus⁹.

Results. We showed previously that isolated granules from the rat or mouse submaxillary gland released their enzyme content when kept at 0°C, but not at room temperature^{3,7}. Of the various agents tested, lauryl alcohol, previously used to stabilize amylase containing granules from the parotid gland⁸, stabilized also the membrane of the granules from the mouse submaxillary gland. When incubated for 30 min in a 0.25 M sucrose solution at 0°C, 74% (68-79) of BAEe esterase activity was released into the supernatant fluid on standing for 20 min from the treated particles. In contrast only 41% (18-51) of the activity was liberated in the presence of 10⁻³ M lauryl alcohol.

The membranes of the granules were further stabilized when, in addition to lauryl alcohol, glutaraldehyde (0.025%) was added to the incubation mixture. The granules so treated lost only 28% (22-41) of the esterase activity into the supernatant fluid on standing, while in these experiments the untreated control released 78% (74-88) after standing for 30 min in an ice bath. Higher concentrations of glutaraldehyde significantly reduced the recovery of enzymic activity.

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When sucrose was replaced by KCl (0.15 M), only 35% of the esterase activity found in the granules was released either in the cold or at room temperature, but only 10% of the activity of untreated control could be recovered from the granules.

The combined use of lauryl alcohol and glutaraldehyde protected granules against lysis in the cold but not against mechanical damage. Millipore filtration broke approximately the same percent of particles in the treated and in the control groups; only 3% to 10% was retained on filters of a pore size of 5 μm or 1.2 μm .

After 4 h of discontinuous density gradient centrifugation the major portions of renin, kallikrein and esterase activities were concentrated between 1.76 M and 1.80 M sucrose solutions showing a maximum at 1.78 M. This distribution pattern of the enzymes was very similar to that obtained previously in a continuous sucrose density gradient⁷.

Rate zonal centrifugation, however, yielded different results. After 40 min of centrifugation at 8,000 g, 3 distinct zones of activities were found: in the 1.1 M, between the 1.2 and 1.3 M and in the 1.5 M sucrose layers. The biggest difference in the distribution of the kallikrein and renin containing granules was found when their relative concentrations in the top and bottom layers were compared. While the top layers (fractions 1+2) contained 55% (52–58) of the renin and 21% (18–24) of kallikrein activity, the lower portions (1.5 M sucrose) had 45% (42–48) of renin and 79% (76–82) of kallikrein after rate zonal centrifugation.

Discussion. Of the enzymic activities assayed, the release of angiotensin by renin and the release of kinin by kallikrein are specific functions, while BAEe can be cleaved by kallikrein and by other enzymes as well². Although most of the esterase activity of the granules from rat gland was due to kallikrein³, other hydrolases contributed to the cleavage of BAEe in the mouse tissue⁷. Thus, we measured the esterase activity of kallikrein to detect the presence of the enzyme, while bioassay of the kinin released by the enzyme from plasma kininogen was used for the more specific determination.

We reported the first separation of kallikrein and renin containing particles intact enough for electronmicroscopic investigations^{3,7}. Electron micrographs of granules obtained from the homogenized submaxillary gland of the mouse revealed a heterogenous population⁷. Some of the granules were large and spherical and resembled the kallikrein containing granules of rat submaxillary gland³ or the zymogen granules of the pancreas⁹. Others were smaller and more amorphous, looking similar to the granules of the juxtaglomerular apparatus of the kidney¹⁰. The results of rate zonal centrifugation experiments reported here were taken as an indication that kallikrein granules had a bigger mean size than the renin storing granules,

since a significant proportion of them sedimented in 40 min, while the relative concentration of renin granules in the more concentrated sucrose layers did not increase. Although this is not decisive evidence, it certainly supports the assumption that the large spherical granules are the ones which contain kallikrein. Rate zonal centrifugation separates the granules primarily according to their sedimentation rate, which depends predominantly on the particle size¹¹.

Isopycnic gradient centrifugation, which separates particles according to their density, showed a parallel distribution of esterase, kallikrein and renin activities. These results are in agreement with the electron microscopic observations that an equally dense population of granules were isolated from the submaxillary gland of the male white mouse⁷.

The membranes of the granules were stabilized against lysis in the cold with lauryl alcohol and glutaraldehyde. Lauryl alcohol protected the membrane against rupture in the cold possibly by combining with a lipid component⁹, while glutaraldehyde cross-linked proteins by coupling to amino groups¹². This combined treatment, however, did not block mechanical damage to the particles during filtration.

Zusammenfassung. Subzelluläre Partikel, die Renin und Kallikrein enthalten, wurden durch Differentialzentrifugation aus homogenisierter Submaxillardrüse der männlichen weissen Maus angereichert. Durch Zonen-zentrifugation wurde die relative Renin-Konzentration in der oberen Schicht des Rohrzucker-Dichtegradienten erhöht, während sich Kallikrein in der unteren Schicht anhäufte. Vorbehandlung der Partikel (granules) mit Glutaraldehyd und Lauryl-Alkohol stabilisierte sie gegen Lyse bei niedrigen Temperaturen.

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Effect of Sodium Aurothiomalate 'Myocrisin' on DNA Synthesis in Phytohaemagglutinin-Stimulated Cultures of Sheep Lymphocytes

It has been shown that the antirheumatoid drugs chloroquine and prednisolone inhibit the stimulatory effect of phytohaemagglutinin (PHA) on lymphocytes in vitro^{1,2}. The purpose of the present investigation was to determine the influence of another antirheumatoid drug, sodium aurothiomalate 'Myocrisin'³, on DNA synthesis in lymphocytes cultured in the presence of PHA.

Intestinal lymph from Dorset horn wethers approximately 18 months old was obtained by cannulating the intestinal lymphatic duct distal to the hepatic duct, using the technique described by LASCELLES and MORRIS⁴. The lymph was collected through a polyvinyl catheter draining into a sterile bottle containing preservative-free heparin (Evans Medical, Liverpool, England). During periods when