will be reported elsewhere[5], amino acid analyses in additional 12 Parkinsonian patients did not show this abnormality of amino acid metabolism. Nevertheless, the possibility that hypoprolinemia represents a biochemical factor in the Parkinsonism of this particular patient cannot be excluded.

Table I. L-Proline in plasma

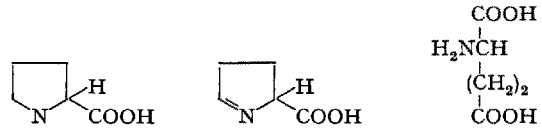
Normal adults	Patient K. M.
$160 \pm 58\ \mu M/l$	18; 11; 16; $25\ \mu M/l$

Means and standard deviations.

Table II. Amino acids in plasma ($\mu M/l$)

Normal adults[a]		Patient K. M.
Glu	69 ± 26	94
Gly	153 ± 43	120
Ala	249 ± 66	296
Val	157 ± 31	177
I Leu	42 ± 11	52
Leu	97 ± 24	85
Tyr	38 ± 8	34
Phe	36 ± 7	37
Lys	105–207[b]	104
His	32– 97[b]	39
Arg	40–140[b]	41
Orn	30– 64[b]	42

[a] Mean from 4 analyses with exception of arginine (3 determinations), runs were done at 4 different occasions within a period of 4 month. [b] The normal values for the basic amino acids are taken from SOUPART[6].



Proline $\rightleftarrows \Delta^1$-pyrroline-5-carboxylate $\rightleftarrows$ glutamic acid.

The biochemical mechanisms responsible for the hypoprolinemia observed are as yet obscure. A defect in the amino acid carrier system appears less probable, because glycine, which is transported by the same carrier system, is not affected. The observations described rather suggest an enzymatic block in the pathway from glutamic acid to proline (Figure), involving either the cyclization step to Δ^1-pyrroline-5-carboxylate or the hydrogenation by the enzyme Δ^1-pyrroline-5-carboxylate reductase[7].

Zusammenfassung. Im Vergleich zu einer Kontrollgruppe zeigten Aminosäurenanalysen im Plasma eines Patienten mit Parkinsonismus eine starke und isolierte Reduktion von L-Prolin.

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Effect of Serotonin Depletion on HC-3-Induced Slow Wave Sleep of Cat

It has been shown in cats, with chronically implanted intraventricular cannulae as well as EEG and EMG recording electrodes, that the 4th ventricular injection of hemicholinium-3 (HC-3) dose dependently increases slow wave sleep (SWS) time[1]. Since HC-3 is a choline transport inhibitor[2,3] and the effect is frequency dependent[4,5], it was rationalized that HC-3 by blocking reuptake of choline prevents resynthesis of acetylcholine (ACh) and lowers the ACh containing neuronal activity which in turn increases SWS, and this is interpreted that brain ACh actively maintains the states of vigilance[1]. This finding, that brain ACh depletion leads to sleep, refutes the concept of a cholinergic hypnogenic system[6].

There is, however, a considerable amount of work which suggests that brain serotonin (5-HT) plays a significant role in SWS. The strongest support of this concept comes from those studies which demonstrates that in cats 5-HT depletion with *para*-chlorophenylalanine (PCPA) leads to insomnia[7–9]. The present study has, therefore, been extended to investigate whether or not brain ACh depletion with HC-3 leads to 5-HT release in the brain which in turn induces SWS. Thus, HC-3 induced SWS time was measured in cats before and after PCPA treatment. If HC-3 ultimately induces SWS by causing 5-HT release then PCPA treatment should block or alter SWS time due to HC-3, since PCPA is a relatively selective 5-HT depletor[10].

Results presented here were collected from cats reported in a previous publication[1] which also reported the methods of surgical preparations and recording arrangements. In brief, each cat was stereotaxically implanted with deep and surface brain electrodes as well as a neck muscle electrode for recording chronically both EEG and EMG in freely moving conditions, and a 4th ventricular cannula

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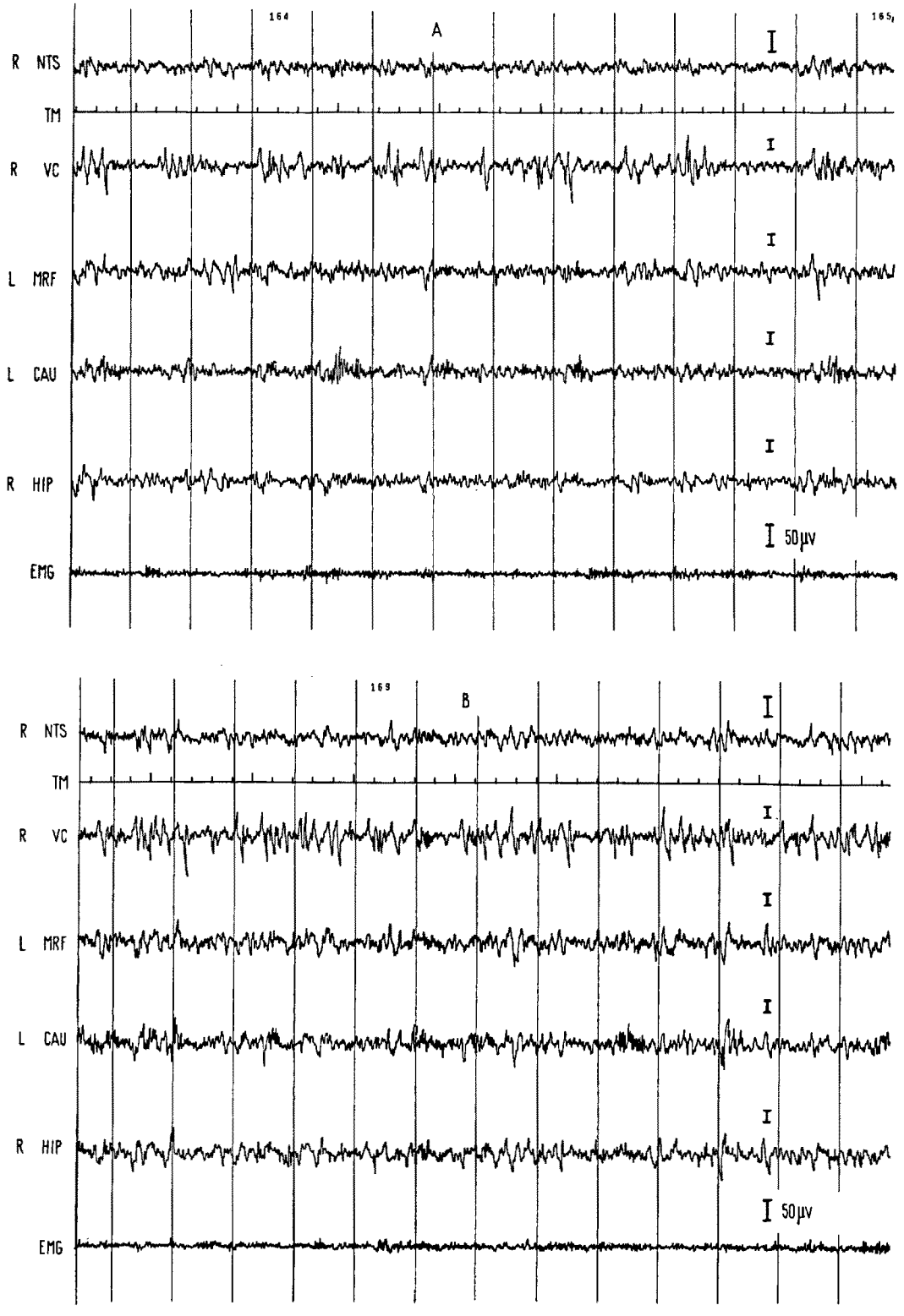
for injecting the drug. Following completion of the investigation, the electrode positions were verified histologically according to a stereotaxic atlas of the cat brain[11]. Three to 4 weeks post-operatively, the cats were placed individually in a light and temperature-controlled, sound-attenuated recording chamber with a one way viewing mirror. In the chamber they had access to food and drink ad libitum. Each recording session lasted between 10.00 to 17.00 h during which EEG and EMG both were recorded on a polygraph (Grass model-7) and corresponding behavioral changes were noted. Each cat was exposed to 2 experimental conditions: 1. After 1 day of acclimatization to the recording arrangement a day of control (day 2) recording followed. On day 3, 5 μg HC-3 dissolved in 50 μl of sterile saline were injected into the 4th ventricle over a period of 60–70 sec; 5 μg were used since in the previous



study[1] this dose of HC-3 effectively facilitated SWS. 5 µg of HC-3 increased 6 h SWS time of cats A, B, and C from 163, 152 and 147 min to 250, 226 and 248 min respectively. There was no qualitative difference in SWS between the control day and HC-3 day. It consisted of both light- and deep-SWS. Light- from deep-SWS was differentiated mainly on the basis of the distribution of high voltage slow waves after a particular segment was classified as a sleep, which was based on EEG, EMG, as well as behavioral notes and is consistent with the methods of URSIN[12] (see also ref. by URSIN). Examples of these 2 types of SWS due to 5 µg of HC-3, before PCPA treatment, are shown in the Figures A and B. 2. The 2nd experimental condition was initiated 15 days after the 1st. Following a day of acclimatization and a day of control recording, each cat was injected at 10.00 h on the next day with PCPA (150 mg/kg, i.p.) which was suspended (100 mg/ml) in sterile steroid suspending vehicle (formula, ref.[9]). As expected from the study of KOELLA et al.[9], 150 mg/kg i.p. PCPA reduced significantly the SWS time of all cats. In the 48 h post-PCPA recording session cats A, B and C demonstrated only11, 0 and 7 min of light-SWS in the 6 h period. 72 h after PCPA treatment the effect of 5 µg of HC-3 administered intraventricularly was reexamined and this time cats A, B and C demonstrated only 76, 25 and 51 min of SWS during the 6 h period. This constitute a 70%, 89% and 80% loss respectively of the HC-3 efficacy to induce SWS when compared to the effect of HC-3 before PCPA treatment, and is statistically significant ($p < 0.01$) by Student's t-test. Furthermore, PCPA pretreatment not only virtually abolished deep-SWS, both during the control recording session (i.e., 48 h post-PCPA) and in the recording session after HC-3 administration in PCPA pretreated animal (i.e., 72 h post-PCPA), but also attenuated light-SWS as evidenced in the background electrical activity level, i.e., increase in low voltage fast activity, especially in the deep areas of the brain. Figure C shows the PCPA induced change in the EEG of light-SWS due to HC-3.

The fact that PCPA treatment causes sleeplessness in all cats supports the contentions of others that brain 5-HT is an essential element in SWS[7–9]. Furthermore, this finding, that pretreatment with PCPA reduces the efficacy of HC-3 to induce SWS, suggests that in the brain a loss in the tonic activity of ACh containing neurons leads to 5-HT release which in turn induces SWS. This is suggested because HC-3 induces SWS by reducing the tone of highly active ACh containing neurons[1]. However, PCPA blocks synthesis of 5-HT[10] and 150 mg/kg i.p. PCPA lowers brain 5-HT level of the cat[9], and pretreatment with 150 mg/kg i.p. PCPA (i.e., brain 5-HT depletion) reduces significantly (70%–89%) the HC-3 (i.e., ACh depletor) efficacy to induce SWS. As brain 5-HT depletion with PCPA treatment reduces the HC-3 efficacy to induce SWS, HC-3 therefore by reducing the tonic activity of ACh

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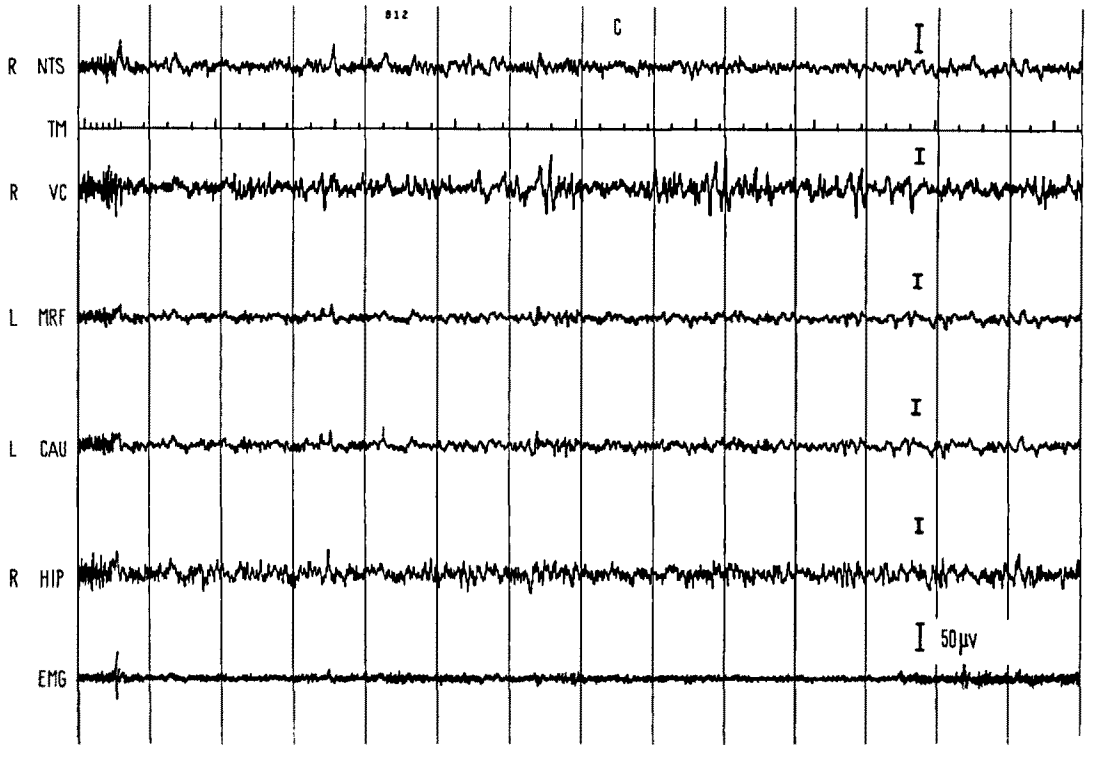

Monopolarly recorded (indifferent electrode, frontal sinus) polygraph record of a cat with chronically implanted electrodes and a 4th ventricular cannula, taken from 2 different recording sessions, each recorded after 5 µg of hemicholinium-3 in 50 µl sterile saline administered intraventricularly over a period of 60–70 sec. A) and B) recorded before *p*-chlorophenylalalnine (PCPA) treatment and shows light slow wave sleep (A) and deep slow wave sleep (B). C) is from the recording session 72 h after 150 mg/kg i.p. PCPA treatment and also shows light slow wave sleep. Note the change caused by PCPA treatment in the EEG which consists of background low voltage fast activity, especially in the deep areas (MRF and CAU) even when the animal is in light slow wave sleep (C). R, right; L, left; NTS, nucleus tractus solitarii; VC, visual cortex; MRF, mesencephalic reticular formation; CAU, Caudate nucleus; HIP, Hippocampus (ventral); EMG, Electromyogram (neck muscle); TM, Time marker, 1 sec.

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 µl 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 Renin[1]

The richest source of kallikrein in the body is the submaxillary gland of the rat[2]. A significant amount of the enzyme in this gland is located in subcellular particles, in granules[3]. The submaxillary gland of the white adult male mouse contains less kallikrein but it has renin as well in large quantities[4–7]. 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 particles[7]. 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 previously[7]. 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 µm.

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-spectrophotometer[3]. 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 rat[7]. Kinin release by kallikrein from a purified human kininogen substrate was followed on the isolated rat uterus[2].

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 gland[8], 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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