Amylase Activity in Laboratory Animal Serum and Tissues

Amylase levels have been determined in the serum of several animals (rat, mouse, guinea-pig, rabbit, cat) and man by the method of WOHLGEMUTH[1,2]. In rat (17 animals), mouse (11) and guinea-pig (13), after a 12 h fast, the amylase activity was about the same, namely 270–300 diastase units (DU) for 1 ml of serum; in cat (6) it was 220 DU, in rabbit (15) 22 DU and in man (6 persons) 10–20 DU for 1 ml of serum.

In 18 rats, kept fasting for 12 h before the experiment, the amylase of submaxillary and sublingual glands was found to be about 20 times higher than that of serum (average = 7224 ± 1100 DU for 1 g of tissue); in parotid glands, amylase was more than 1000 times higher than that in serum and more than 50 times higher than that in submaxillary and sublingual glands (average = 362,000 ± 54,000 DU for 1 g of tissue).

In liver, amylase was about 40 DU for 1 g. After a 48 h fast, the amylase level in rats (14) was lower than after a 12 h fast, but the ratio between the respective data of the two groups was the same. In 12 h fasted rats (14), operated on 6 days before for removal of the salivary (submaxillary, sublingual and parotid) glands, the serum and the liver amylase were about the same as in non-operated rats.

The data reported here are not entirely in agreement with the results of SCHNEYER and SCHNEYER[3], according to whom amylase activity of submaxillary and sublingual rat glands (as in our experiments, the small sublingual lobe was not separated from the submaxillary gland) is significantly lower – approximately between 3–9% (only occasionally 50%) – than that of the serum and also lower than that of the liver.

In 19 rats, hepatectomized 42 h before through surgical removal of 65–75% of the hepatic parenchyma[4], and 12 h fasted, the serum amylase was the same (13 rats) or only a little lower (6 rats) than that of control rats not hepatectomized.

These results suggest that serum amylase levels, at least in the rat, do not depend on the salivary glands or the liver; therefore in the rat, the serum amylase also seems to be chiefly from a pancreatic source.

Riassunto. Si è studiato il tasso amilasico nel siero di vari animali, nonchè nelle ghiandole salivari e nel fegato di ratto. L'amilasi sierica non varia significativamente nel ratto privo delle ghiandole salivari o di 2/3 del fegato.

G. M. CARMINATI and M. CATTORINI

Laboratori di Farmacologia della Società Italiana Prodotti Schering, Milano (Italia), April 8, 1964.

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LEÃO's Spreading Depression in the Thalamic Nuclei of Rat

The cortical spreading depression (SD) of LEÃO[1] was recently described in different cortical and subcortical structures (hippocampus, striatum, cerebellar cortex, amygdala – MARSHALL[2], BUREŠ[3], OCHS[4], FIFKOVÁ[5], FIFKOVÁ[6]). Continuity of grey matter seems to be a necessary prerequisite of SD which does not penetrate across compact white matter bands. The purpose of the present paper was to answer the question whether it is possible to evoke SD in a non-homogeneous structure, divided by the white matter laminae into a number of nuclei, typically represented by the system of thalamic nuclei.

Slow potential changes, used as the main indicator of SD, were led off with saline filled capillaries (300 μ) connected to wick calomel cell electrodes. The steady potential change was recorded with a six-channel recording millivoltmeter. SD was elicited by intracerebral microinjection of 0.5 μl of KCl (25% solution). In 7 rats the injection needle was introduced to thalamic points +2.5, 2, 5.5 and 7.5, while three glass capillaries (with 1 mm intercapillary distance) were inserted into the same frontal plane (+2.5) 5–7 mm deep. In another group of 9 rats, the injection needle and one capillary electrode were introduced into the right thalamus (+2.5, 2, 5.5 and +2.5, 1, 5 respectively). On the left side, an array of three capillaries connected with the second injection needle was inserted in the parasagittal plane 1.5–2.5 mm from the midline. Whereas the depth of the electrode system on the right side was not changed during the experiment, capillaries in the left hemisphere were inserted in 2–3 one-millimeter steps (Figure). The location of the electrode positions was checked in Nissl stained sections.

Characteristic SD waves could be elicited in this way from different thalamic loci. The average amplitudes of the slow negative waves obtained from these structures and their average duration calculated to 50% of maximum negativity are summarized in the Table. The spreading velocity was 2.42 ± 0.21 mm/min (n = 96). No spread of thalamic SD was found to hypothalamic regions, neither could SD be elicited by KCl injection into the hypothalamus (area hypothalamica lateralis). In the capsula interna adjacent to the lateral part of thalamic nuclei, low waves of negativity attaining not more than 3 mV could be registered. Significant decrease in the slow potential change amplitude was found between the groups of midline and medial nuclei and the lateral and

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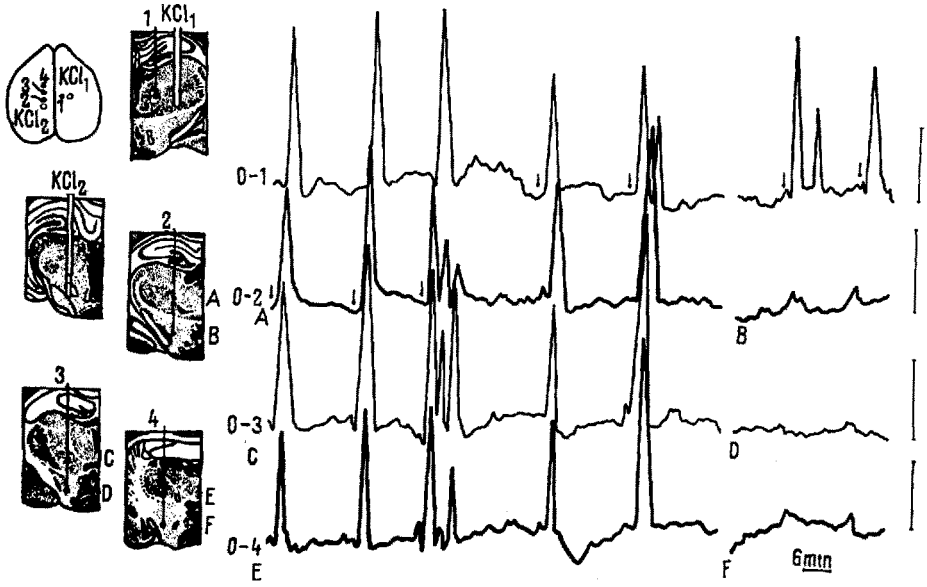
Slow potential changes of spreading depression in thalamic nuclei

			Thalamus												Hypothalamus		
			M						L								
			MD	NCM	NPC	PVP	RE	VM	GL	GM	PTH	R	SG	VE	VDM	AHL	HDM	ZI
% of positive reactions in KCl application	Homo-lateral	M		100 n=7			100 n=7	100 n=3		100 n=2		100 n=6						
		L	100 n=14				100 n=2	100 n=2	22.2 n=9	90 n=20	60 n=5	50 n=4	75 n=4	52 n=19	100 n=26	0 n=2	0 n=1	0 n=2
	Contra-lateral	M	60 n=10	100 n=3	100 n=2			100 n=9		50 n=4		0 n=1		83 n=12	75 n=4			
		L	100 n=13			0 n=6			33.4 n=3	22.2 n=9		66.6 n=6		50 n=8		0 n=2		0 n=3
Amplitude (mV)			7.8 ± 0.3 n=72	10.4 ± 0.8 n=22	6.0 ± 0.03 n=5		8.0 ± 1.5 n=13	9.2 ± 0.7 n=28	4.4 ± 0.7 n=4	6.6 ± 0.6 n=44	5.1 ± 1.2 n=4	6.4 ± 0.7 n=16	4.7 ± 1.5 n=5	8.3 ± 0.6 n=53	7.6 ± 0.5 n=65			
	Mean value		8.5 ± 0.3 n=140						7.3 ± 0.3 n=191									
Duration of the negative wave (sec)			61.2 ± 3.1 n=71	58.5 ± 5.6 n=22	57.8 ± 1.5 n=5		64.4 ± 10.4 n=13	54.3 ± 4.6 n=27	72.2 ± 9.9 n=4	47.8 ± 2.7 n=44	78.8 ± 4.5 n=4	53.0 ± 6.0 n=16	43.2 ± 2.4 n=5	52.0 ± 2.5 n=53	57.6 ± 3.2 n=63			

M = group of nuclei of midline + medial group. L = lateral + posterior group.

AHL – Area hypothalamica lateralis; GL – Geniculatum laterale; GM – Geniculatum mediale; HDM – Nucleus hypothalamicus dorsomedialis; MD – Nucleus dorsomedialis; NCM – Nucleus centralis medialis; NPC – Nucleus paracentralis; PTH – Nucleus posterior; PVP – Nucleus paraventricularis posterior; R – Nucleus reticularis; RE – Nucleus reuniens; SG – Nucleus supra-geniculatus; VE – Nucleus ventralis; VM – Nucleus ventromedialis; VDM – Nucleus ventralis pars dorsomedialis; ZI – Zona incerta.



Recording of slow potential changes accompanying SD in the thalamus of rat. 0–1 Nucleus dorsomedialis; 0–2 A nucleus ventromedialis, B area hypothalamica lateralis; 0–3 C nucleus ventralis pars dorsomedialis, D area hypothalamica lateralis; 0–4 E nucleus ventralis, F area hypothalamica lateralis. Arrows indicate KCl application. The reference electrode (O) was placed on neck muscles. Calibration 5 mV.

posterior group. Also the incidence of positive SD reactions in those parts of the thalamus was decreased (Table).

Thus SD can be more readily elicited in the medial part of the thalamus than in the lateral and posterior ones, perhaps because of the fibres penetrating these regions and joining the capsula interna rostrally and lamina medullaris externa occipitally. The impaired transition of SD from the medial to the lateral thalamus may be caused by the lamina medullaris interna enveloping the medial thalamic complex.

Zusammenfassung. Mit KCl-Microinjektion werden im Thalamus langsame Potentialwellen hervorgerufen, deren Amplitudendauer und Ausbreitungsgeschwindigkeit der «spreading depression» von LEÃO (SD) entspricht. Die Bedingungen für SD-Entwicklung sind infolge grösserer Zelldichte in der Gruppe der medialen thalamischen Kerne besser als in den lateralen und posterioren Kernen, was sich in der verminderten Amplitude des langsamen Potentials und in häufigem Wegfall einer positiven Reaktion zeigt.

EVA FIFKOVÁ

Institute of Physiology, Czechoslovak Academy of Sciences, Prague (Czechoslovakia), May 15, 1964.

Production of Reddish-Brown Pigment from *dl*-Tryptophan by Enterobacteria of the Proteus-Providencia Group

Oxidative deamination of α-amino acids is one of the typical biochemical reactions of the Proteus-Providencia group[1]. The resulting pyruvic acids, especially in the case of cyclic amino acids such as phenylalanine, tryptophane, histidine etc., produce with $FeCl_3$ coloured solutions. This reaction is utilized for the so-called phenylalanine test in routine diagnostic practice[2,3].

Studying the above-mentioned reaction in the basal medium used for phenylalanine test[4], in which this amino acid was replaced by *dl*-tryptophane, the authors observed that in the medium inoculated with bacteria of the Proteus-Providencia group a reddish-brown pigment diffusing in the medium developed after 12 h incubation at 37°C, even though the medium was not treated with $FeCl_3$ solution. As far as the authors know, this phenomenon has not been recorded in the literature. By further incubation (24–48 h) the intensity of the colour increased. Other cyclic amino acids failed to give this production of reddish-brown pigment and it was specific only for the above-mentioned group of bacteria. Therefore a detailed examination of this newly observed phenomenon was carried out.

In order to confirm that the reddish-brown pigment is produced only by bacteria of the Proteus-Providencia group from the *dl*-tryptophane, the authors compared production of the pigment in strains of the Proteus-Providencia group with those from other bacterial genera (all cultures obtained from culture collection), and the characteristic was also examined in 2095 strains of Gram negative bacteria isolated from clinical material; these were mostly enterobacteria, according to their biochemical characteristics, among which the classical phenylalanine test was included. From the total of 41 bacterial

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