Effects of Sleep Dialysate, Substance P and Psychotropic Drugs upon the Thalamo-Cortical Evoked Response

Dialysis of cerebral blood performed upon rabbits during sleep induced by electrical stimulation of the hypnogenic thalamic system provides a dialysate which when later injected into a recipient rabbit elicits moderate sleep[1,2]. Control dialysate obtained by dialysis from animals in which sham stimulation replaces electrical stimulation of the thalamus does not induce sleep. In the present experiment the potentials evoked in the motor cortex by electrical stimulation of the medio-central intralaminary thalamus were utilized as criteria to compare the effects of sleep dialysate with control dialysate, substance P (SP), chlorpromazine and amphetamine. SP (28 U/mg protein) was a preparation made by the new method of LEMBECK, HEIZMANN and SEIDEL from pigs brain. The evoked potential findings were correlated with electrophysiological and behavioral data.

According to TISSOT and MONNIER[3,4] single shocks or low frequency stimulation applied to the hypnogenic thalamic area of the rabbit induce a first short latency surface positive non-recruiting component followed by a second longer latency surface negative recruiting response. These responses appear to behave antagonistically to each other so that as one increases in amplitude the other diminishes[3–6]. Thus, for example central activating drugs such as amphetamine, nikethamide (coramine) or LSD increase the first component and decrease the second, whereas central moderating drugs such as morphine, chlorpromazine or reserpine reverse the process. To these components of the cortical evoked response may be added, at least if stimulation is presented at about 3 per sec, a third, longer latency, large surface negative wave whose amplitude appears to parallel that of the second or recruiting response.

Methods. The effects of sleep dialysate, control dialysate, SP, chlorpromazine or amphetamine were tested on each of 28 unanaesthetized rabbits. After habituating an animal to the experimental surroundings for 1 h subsequent to implanting electrodes in accordance with the technique of MONNIER and GANGLOFF[7], 20 min of spontaneous electrical cortical activity was monitored on a 16 channel Schwarzer oscillograph in conjunction with a Faraday automatic frequency-amplitude wave analyser. Subsequently, 3 per sec, 3 msec pulse duration bipolar electrical stimulation of the medio-central intralaminary thalamus was provided by a Grass model S4 stimulator, and the potentials elicited were recorded monopolarly from the motor cortex.

Beginning below threshold, the voltage was augmented in increments of 0.25 V until bilateral cortical recruiting responses were observed on the oscillograph. The criterion for recruiting was waxing and waning of the second component (surface negative) of the thalamo-cortical evoked response in such a fashion that the amplitude was small at the onset of stimulation, increased to a maximum after three or four stimuli and then diminished, the entire process again repeating itself. After a threshold was obtained 20 successive evoked potentials were recorded from the motor cortex by means of a Mnemotron computer of average transients (CAT) at 0.25 V above threshold, and this record was then photographed.

Immediately afterwards a test substance was injected. Spontaneous electrocorticographic (ECoG) activity was recorded for most of the next hour with interruptions occurring after 15 min, 30 min and 60 min to permit evoked responses to be recorded. At these times thresholds were determined and then 20 successive evoked responses were recorded on the computer using stimulation parameters identical with those employed during the preinjection baseline period. Since the thalamo-cortical threshold rarely increased by more than 0.25 V after the baseline period, and since the original computer recording was taken at 0.25 V above threshold, an adequate computer analysis was almost invariably obtained.

Results. Evoked potentials: Photographed computer records were used to determine the latencies and durations of each component of the evoked response. The first component had a latency of 4–7 msec, a duration of 5–10 msec, and was generally but not invariably surface positive. Following this component a surface negative recruiting component occurred which had a latency of 9–17 msec and a duration of 25–35 msec. A slow surface negative wave at a latency of 44–56 msec and a duration of 100–150 msec was frequently observed except in the records of very aroused animals. Examples of form and amplitude changes of the thalamo-cortical evoked response as a function of the test substances over time may be seen in Figure 1.

In general the thresholds of thalamo-cortical evoked responses decreased after injection of sleep dialysate (20 ml, i.v.), SP (75 U/kg of 28 U/mg, i.v.) or chlorpromazine (5 mg/kg, i.m.). The decrease in threshold after administration of chlorpromazine became particularly pronounced approximately 15 min after injection. Within 30 min after injection of chlorpromazine or SP thresholds increased towards the preinjection baseline level, whereas they remained lowered longer after administration of sleep dialysate. Control dialysate (20 ml, i.v.) either did not alter the threshold or increased it; and amphetamine (1 mg/kg, i.v.) usually elevated the threshold.

The changes in amplitude of the recruiting component of the thalamo-cortical response after injection are shown for each substance in Figure 2. Sleep dialysate, SP and chlorpromazine depressed the first surface positive component and enhanced the second and third surface negative components. The augmentation of the second or recruiting component was a more reliable phenomenon than the change in either the first component or the negative slow wave. Under control dialysate or amphetamine the first component was often augmented, whereas the second and third parts of the response were depressed. The decreases in amplitude of the recruiting component and slow wave were more pronounced under amphetamine than under control dialysate, but the effects of both substances were consistent with concurrent ECoG and behavioral manifestations of increased wakefulness. The arousal observed in control dialysate injected animals was probably partly due to cumulative effects of restraints placed upon them. In the cases of sleep dialysate, SP and

[1] M. MONNIER and L. HÖSLI, Science *146*, 796 (1964).
[2] M. MONNIER and L. HÖSLI, Pflüger's Arch. ges. Physiol. *282*, 60 (1964).
[3] R. TISSOT and M. MONNIER, Helv. physiol. pharmacol. Acta *16*, c33 (1958).
[4] R. TISSOT and M. MONNIER, EEG clin. Neurophysiol. *11*, 675 (1959).
[5] M. MONNIER, L. HÖSLI, and P. KRUPP, EEG clin. Neurophysiol. Suppl. *13*, 97 (1963).
[6] M. MONNIER, M. KALBERER, and P. KRUPP, Exp. Neurol. *2*, 271 (1960).
[7] M. MONNIER and H. GANGLOFF, *Rabbit Brain Research*, vol. I (Elsevier, Amsterdam 1961).

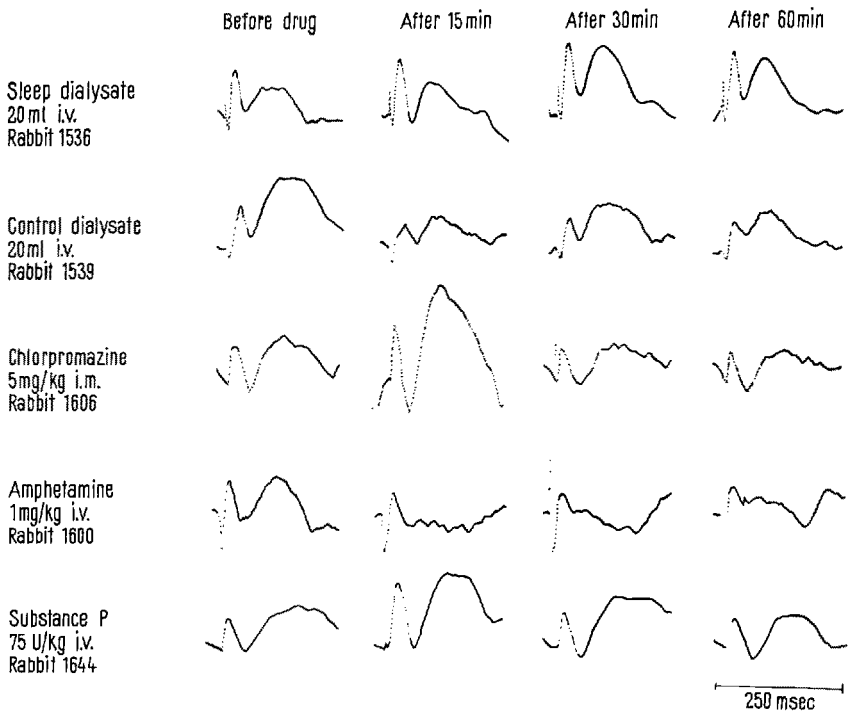

Fig. 1. Evoked potentials elicited from the medio-central intralaminary thalamus (3 per sec, 3 msec pulse duration bipolar electrical stimulation), and recorded monopolarly from the motor cortex. Each frame represents 20 successive evoked responses summed by a Mnemotron computer (CAT). Note the marked increase of the second, surface negative recruiting component 15 min after injection of sleep dialysate, substance P and chlorpromazine in contrast with the decreases after administration of control dialysate and amphetamine.

chlorpromazine these waking tendencies were counteracted by hypnogenic effects. A t-test comparison between sleep and control dialysate groups indicated a reliable difference ($p < 0.05$) in mean amplitude change of the recruiting component during the 60 min following injection.

Electrographic visceral and somatic findings: The ECoG and behavioral alterations were generally consistent with the evoked potential picture. Under sleep dialysate, SP or chlorpromazine cortical delta activity and number of spindles increased, whereas episodes of cortical desynchronization decreased in duration. The onset of these changes occurred within 5 min after administration of SP or chlorpromazine, but required about 10 min after injection of sleep dialysate. Diminution of muscle tonus, drooping of the ears and partial eye closures also occurred in these groups. After injection of SP or chlorpromazine ECoG, and behavioral effects reached their maximum within 15 min and then dissipated rapidly, leaving what appeared to be alert but relaxed animals. In contrast, sleep dialysate effects often did not reach their peak until about 30 min after injection.

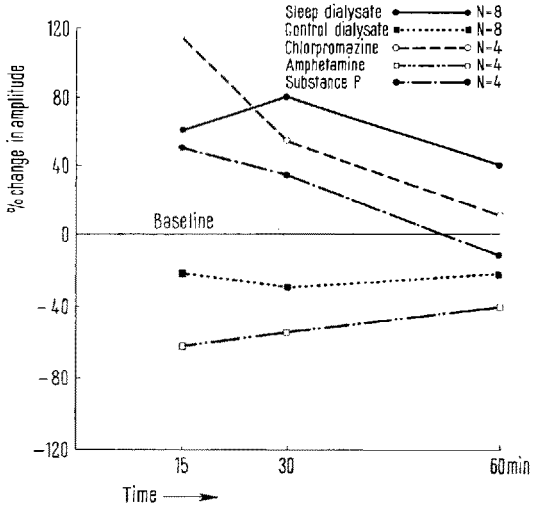

Fig. 2. Percentage changes in amplitude of the recruiting component of the thalamo-cortical evoked response for the 60 min following injection. Each point represents the comparison of 20 successive computer (CAT) summed responses before and after injection of the test substance.

Unlike sleep dialysate, SP and chlorpromazine injected animals, and rabbits receiving control dialysate or amphetamine revealed decreased delta and spindle activities and longer episodes of cortical desynchronizations. Increased muscle tonus and tendencies towards escape behavior were also observed. In all groups except amphetamine, heart rate remained stable, although with increased dosages of chlorpromazine bradycardia occurred. Respiration rates in sleep dialysate, control dialysate and SP groups also remained stable, whereas amphetamine often caused a marked augmentation and chlorpromazine a pronounced diminution of respiration rate that frequently persisted throughout the experiment.

In the present investigation the recruiting-component of the thalamo-cortical evoked response appeared to be highly correlated with ECoG and behavioral alterations as criteria of hypnogenic effects. According to each of these criteria sleep dialysate, SP[8] and chlorpromazine

[8] F. Lembeck, A. Heizmann, and G. Seidel, Biochem. Pharmacol., in press.

resembled one another in moderating effect, but differed considerably from control dialysate or amphetamine[9,10].

Zusammenfassung. Die Wirkung des Schlafdialysates des Kaninchens auf das Gehirn eines normalen Empfängers kennzeichnet sich durch Erregbarkeitszunahme des mediozentralen somnogenen thalamischen Feldes (Amplitudenzunahme der im Cortex ausgelösten Potentiale). Eine ähnliche Wirkung übt Chlorpromazin als zentraldämpfendes Pharmakon und Substanz P aus, während zentrale Stimulantien wie Amphetamin eine umgekehrte Wirkung entfalten.

N. SCHNEIDERMAN[11], M. MONNIER[12], and F. LEMBECK

Physiologisches Institut der Universität Basel (Switzerland), July 26, 1965.

[9] The substance P was provided by the Department of Pharmacology, University of Tübingen (Germany).

[10] This study was supported by grants from 'Ciba-Stiftung', Hoffmann-La Roche AG Basel, and Sandoz AG Basel.

[11] Present address: Department of Psychology, University of Miami (Florida USA).

[12] Requests for reprints should be sent to Prof. M. MONNIER, Physiologisches Institut, Vesalgasse 1, Basel (Switzerland).

Mucopolysaccharides in Experimental Hepatic Fibrosis

Chronic administration of hepatotoxic agents induces hepatic sclerosis in the rat, which is generally well established after 100 days. At the beginning of this pathologic change one can see the accumulation of an extracellular material, which gives histochemically a positive periodic acid-Schiff reaction and displays metacromasia after staining with toluidine blue. The mucopolysaccharide nature of this substance is now generally accepted according to the findings of POPPER et al.[1]. The possible role of this material in hepatic fibrogenesis led us to study the biochemical modifications of mucopolysaccharide constituents during chronic carbon tetrachloride intoxication in rat.

14 Wistar male rats (300–400 g) received subcutaneous carbon tetrachloride (0.15 ml in 0.5 ml mineral oil) twice weekly over a period of 3 months. The animals were allowed a regular diet. At the end of the experiment the 14 experimental and 5 control animals were killed, the liver immediately homogenized at 0°C, delipidized[2] and dried. The following quantitative determinations were then performed: hydroxyproline[3]; total hexosamines[4]; extraction of[5] and determination of hexuronic acids[6]; and total protein bound sulphates[7]. Our results are summarized in the Table.

The following points may be emphasized: (1) At the end of the 3-month period of intoxication, micronodular fibrosis was macroscopically visible and the accumulation of collagen in the fibrotic livers was marked. This was demonstrated by a fivefold increase in the hydroxyproline content of damaged livers as compared to the normal ones. (2) The total hexosamine content was increased by 79% and protein bound sulphates by 94% of the normal values. The differences between these averages and those of the controls are very significant. (3) In contrast with these obvious changes, the concentration of hexuronic acids in the pathologic livers was practically the same as that in the normal livers. The high value of the correlation coefficient (Figure) between the hexosamine concentration and the protein bound sulphates in the pathologic livers is evidence that the hexosamine-rich material is sulphated.

[1] H. POPPER, F. SCHAFFNER, H. HUTTERER, N. PARONETTO, and F. BARKA, Ann. New York Acad. Sci. *86*, 1075 (1960).
[2] W. C. SCHNEIDER, J. biol. Chem. *161*, 293 (1945).
[3] H. J. KIVIRIKKO and F. LIESMAA, Scand. J. clin. lab. Invest. *2*, 128 (1959).
[4] N. F. BOAS, J. biol. Chem. *204*, 553 (1953).
[5] A. J. BOLLET, J. clin. Invest. *37*, 858 (1958).
[6] T. BITTER and H. M. MUIR, Anal. Biochem. *4*, 330 (1962).
[7] K. S. DODGSON, Biochem. J. *78*, 312 (1961).

Mean values and standard deviations of hydroxyproline, total hexosamine, total hexuronic acid and protein-bound sulphate concentration in dried de-fatted liver of normal and CCl_4-treated rats

	Hydroxyproline	Total hexosamines	Total hexuronic acids	Protein-bound sulphates
	mg/100 g	mg/100 g	mg/100 g	mg/100 g
Normal (5)	108.04 ± 14.29	441.66 ± 34.40	144.33 ± 18.52	72.32 ± 12.97
CCl_4-treated (14)	510.51 ± 142.04	794.89 ± 105.38	136.14 ± 28.57	164.80 ± 54.75
't'	4.77	5.93	0.015	2.64
p	$\ll 0.001$	$\ll 0.001$	> 0.90	$0.02 > p > 0.01$

't': significance test. p: significance level.