result of Peterson et al. obtained in dogs can also be ascribed to a direct access of reabsorbed glucose to renal capsular lymph.

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2 J.C. Pedersen, E.G. Persson and A.B. Maunsbach, in: Functional Ultrastructure of the Kidney, p. 443. Ed. A.B. Maunsbach, Olsen and E.L. Christensen. Academic Press, London 1980.
3 W. Pfaller and M. Rittinger, Mikroskopie *33*, 74 (1977).
4 W. Kriz and P. Napiwotzky, Contr. Nephrol. *16*, 104.
5 W. Kriz and H.J. Dieterich, Z. Anat. EntwGesch. *131*, 111 (1970).
6 K.H. Albertine and C.C.C. O'Morchoe, Kidney int. *16*, 470 (1979).
7 D.R. Bell, G.G. Pinter and P.D. Wilson, J. Physiol., Lond. *279*, 621 (1978).
8 C.R. Rao, in: Linear Statistical Inference and its Applications, p. 136. Wiley, New York 1965.
9 T.V. Peterson, B. Benjamin, E.M. Hasser and M.J. Keyl, Invest. Urol. *16*, 131 (1978).

Cholinergic mechanisms in the production of focal cortical slow waves[1]

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Summary. Microiontophoretic application of scopolamine and atropine usually induced or increased focal cortical slow waves of under 3 Hz and abolished or decreased focal fast waves of over 6 Hz whereas acetylcholine iontophoresis and electrical stimulation of the mesencephalic reticular formation had the opposite effect, suggesting that focal cortical slow waves may be due to the interruption of cholinergic input from the reticular formation.

The mechanisms which produce focal slow waves in the EEG are poorly understood. Because focal slow waves appear at the site of lesions which destroy white matter underlying the cortex, it has been suggested that these slow waves are due to partial cortical deafferentation[3,4]. The observation that systemic administration of atropine, a blocker of muscarinic cholinergic receptors, induces generalized slow waves[5-7] has led to the suggestion that slow waves may result from an interruption of normal cholinergic input to the cortex[8]. Since slow waves can be suppressed by electrical stimulation of the midbrain reticular formation (MRF)[9] which projects to the cortex at least in part through cholinergic fibers[10], one may ask whether focal slow waves may be due to the interruption of cholinergic input from the MRF to the cerebral cortex. This idea finds some support by experiments showing that injection of atropine into cortical arteries causes local slow waves which are reduced by stimulation of the MRF[11]. Because systemic applications of atropine cannot determine the site of drug action and because topical application of atropine to the cortical surface causes local spikes[12], we attempted to answer this question by studying the effects of microiontophoretic application of acetylcholine (ACh) and its antagonists on the local cortical EEG and by comparing these effects with that of MRF stimulation.

22 cats were prepared under inhalation anesthesia with a mixture of oxygen, nitrous oxide and halothane. The spinal cord was transected at C1 and the animal was ventilated with an air-oxygen mixture. Wound margins and pressure points were infiltrated with local anesthetic. Blood pressure, heart rate, temperature and CO_2 level were monitored. Eight-barreled micropipettes with tip diameters of 3–5 µm and with protrusions of the central recording tip of up to 60 µm[13] were used. The recording barrel contained potassium citrate (2 M, pH 7), the surrounding barrels contained scopolamine hydrobromide (1 M, pH 5.5), atropine sulfate (0.5 M, pH 7.3), ACh chloride (1 M, pH 4.3) and sodium chloride (3 M, pH 7). Drugs were expelled for periods of 32–64 sec with currents of usually 5×10^{-8} A and retained with currents of opposite polarity up to 1×10^{-8} A between applications. A bipolar stimulating electrode was inserted into the MRF using a stereotaxic atlas[14] and a frame of David Kopf Instruments (A 3 to 4, L 3 to 5, D 0 to −2). The location of the stimulus electrode was verified postmortem.

The micropipette was inserted into the pericruciate cortex. The EEG was recorded from tip positions at depths of up to 2 mm for at least 1 min before, during and after microiontophoretic drug applications. The EEG was also recorded during periods of intermittent stimulation of the MRF with electrical pulses of 0.01 msec and up to 25 V, delivered in trains of 30 stimuli at 300 Hz; the trains were repeated every 2–5 sec. Recordings were stored on magnetic tape and later filtered to eliminate neuronal action

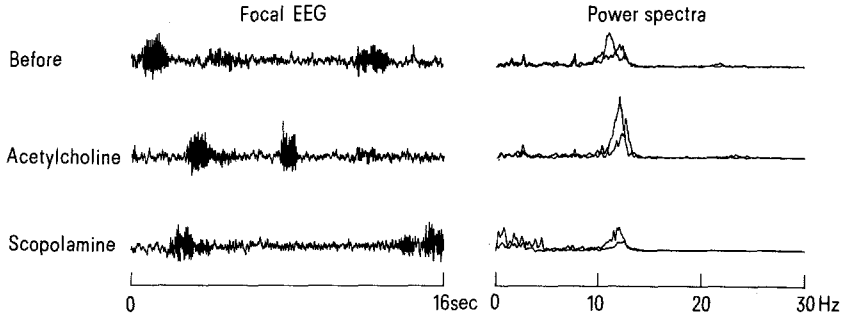

Figure 1. Left: Focal EEG at the micropipette tip during 16 sec of recording before drug application, during iontophoretic application of acetylcholine (5×10^{-8}A) and of scopolamine (5×10^{-8}A). Right: Power spectra computed for 32-sec epochs of EEG recording; 2 power spectra are superimposed for each condition.

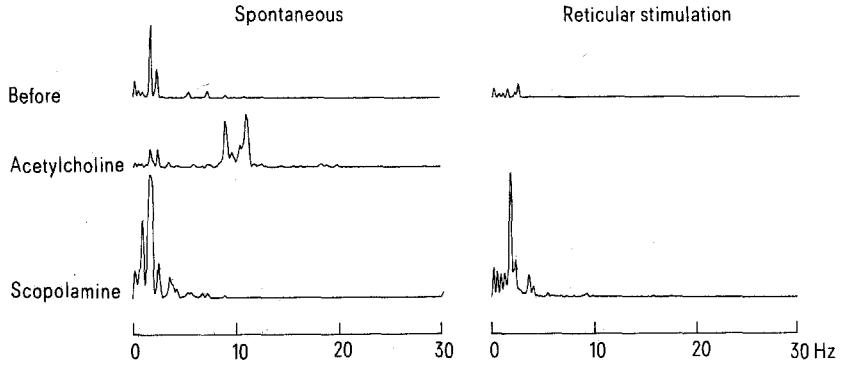

Figure 2. Left: Power spectra of 32 sec of spontaneous EEG recorded at the micropipette tip before drug application, during application of acetylcholine (10×10^{-8} A) and of scopolamine (5×10^{-8} A). Right: Power spectra of the intracortical EEG recorded at the microelectrode tip during 32 sec of intermittent stimulation of the mesencephalic reticular formation.

potentials. Power spectra were computed for epochs of 32 sec with a Nicolet Instrument Company computer (Med-80) and integrated over frequency. Slow waves were defined as waves of less than 3 Hz and fast waves as waves of over 6 Hz; increase and decrease of power was defined as a change of over 25% of the power in these frequency bands during the control recording.

1. *Scopolamine* was applied iontophoretically in 40 electrode positions and induced (fig. 1) or increased (figs 2 and 3) slow waves, decreased fast waves, or had both effects (fig. 1) in 21 (53%) positions. An increase of fast waves or a decrease of slow waves occurred in only 4 instances (10%). Scopolamine was without effect in 15 electrode positions (38%). The effect of atropine iontophoresis was similar in direction but more marked in intensity than that of scopolamine. Figure 3 shows a quantitative evaluation of the power of waves of different frequencies and indicates a greater increase of very slow waves during atropine than during scopolamine application.

2. *Acetylcholine* iontophoresis induced (fig. 2) or increased (fig. 1) fast waves, reduced slow waves, or had both effects in 17 of 40 (43%) electrode positions. Opposite effects were seen in 9 positions (23%), and ACh had no effect in 14 (35%). In most electrode positions, both scopolamine and ACh were effective and produced power shifts in opposite directions although in some positions only one agent was effective. In a few positions, power shifted in the same direction during application of each agent. Overall, the direction of power shifts induced by scopolamine and ACh differed significantly from each other ($p < 0.01$) in Fisher's exact test.

3. *Stimulation of the MRF* reduced slow waves (fig. 2, top right versus left), increased fast waves, or had both effects in 10 of 22 electrode positions (45%) and had the opposite effect or no effect in the remainder. Stimulation of the MRF reduced the slow waves induced by scopolamine (fig. 2, bottom right versus left). In most cases, stimulation of the MRF had the same effect as did ACh, and an effect opposite to that of scopolamine. In some positions, only one or two of the three variables were effective, and in a few cases, MRF stimulation had the same effect as did scopolamine or an effect opposite to that of ACh. Overall, the effect of MRF stimulation differed significantly from that of scopolamine ($p < 0.01$) and did not differ significantly from that of ACh ($p = 0.25$) in Fisher's exact tests.

Our results show that muscarinic cholinergic agonists and antagonists can respectively increase and decrease the frequency of local EEG waves by a direct action on the cerebral cortex. The finding that the antagonists were effective more often than was ACh might have been due to the presence of a continuously active cholinergic input to cortical neurons which could be blocked by the antagonists but could not be further activated by the addition of ACh.

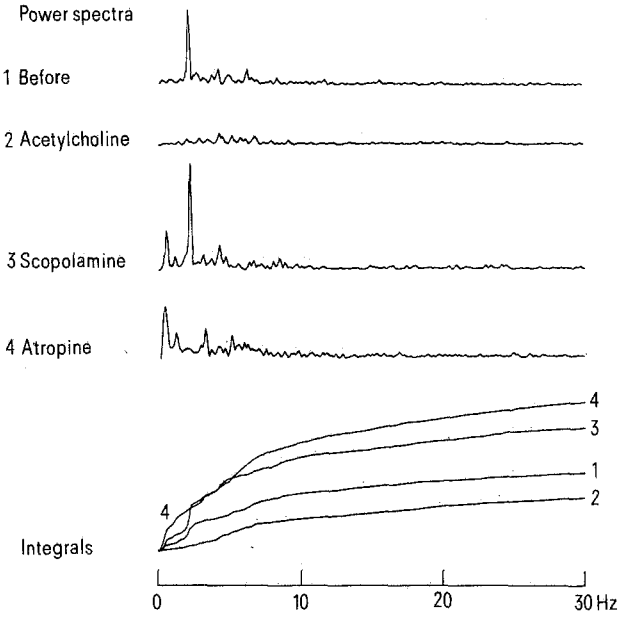

Figure 3. Power spectra of 32 sec of intracortical EEG recording before and during sequential application of acetylcholine (5×10^{-8} A7, scopolamine (5×10^{-8} A) and atropine (5×10^{-8} A). Integrals of power spectra during each condition are shown at the bottom.

These local drug effects show that a blockade of muscarinic cholinergic cortical receptors can produce focal slow waves in the EEG but do not indicate the role of cholinergic mechanisms in the production of the normal and the pathological EEG. However, our finding that MRF stimulation had an effect which was similar to that of ACh, opposite to that of the antagonists, and directly antagonized the simultaneous application of ACh antagonists would support the notion that local slow waves may be caused by interruption of cholinergic input from the MRF. Because projections from the MRF are distributed widely through the hemispheres, this mechanism of focal slow wave production may be very common in cases of structural lesions involving subcortical white matter.

The neuronal mechanism by which interruption of cholinergic transmission produces slow waves is not known. It is possible that blockade of a cholinergic input increases the efficiency of postsynaptic potentials at individual neurons or facilitates the synchronization between them and thus increases the duration and the summation of the electrical events forming the local EEG.

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2 We thank Mr C.C. Smathers, Jr, for his technical help.
3 J.F. Hirsch, J. Buisson-Ferey, M. Sachs, J.C. Hirsch and J. Scherrer, Electroenceph. clin. Neurophysiol. *21*, 417 (1966).
4 P. Gloor, G. Ball and N. Schaul, Neurology, Minneap. *27*, 326 (1977).
5 A. Wikler, Proc. Soc. exp. Biol. *79*, 261 (1952).
6 F. Rinaldi and H.E. Himwich, Archs Neurol. Psychiat. *73*, 396 (1955).
7 P.B. Bradley and J. Elkes, Brain *80*, 77 (1957).
8 N. Schaul, P. Gloor, G. Ball and J. Gotman, Brain Res. *143*, 475 (1978).
9 G. Moruzzi and H.W. Magoun, Electroenceph. clin. Neurophysiol. *1*, 455 (1949).
10 R. Spehlmann and K. Downes, Brain Res. *74*, 229 (1974).
11 F. Rinaldi, Prog. Brain Res. *16*, 229 (1965).
12 J.C. Daniels and R. Spehlmann, Electroenceph. clin. Neurophysiol. *34*, 83 (1973).
13 R. Spehlmann, Electroenceph. clin. Neurophysiol. *27*, 201 (1969).
14 H.H. Jasper and C. Ajmone-Marsan, A stereotaxic atlas of the diencephalon of the cat. The National Research Council of Canada, Toronto 1954.

Lack of effect of cortisone, thyroxine and insulin on the developmental pattern of mouse intestinal glucose-6-phosphatase activity[1]

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Summary. Daily administration for 3 days of cortisone (25 µg/g b.wt), thyroxine (1 µg/g b.wt) or insulin (12.5 mU/g b.wt) to 8-day-old suckling mice does not induce a premature decrease of the phosphohydrolase activity of intestinal glucose-6-phosphatase.

Glucose-6-phosphatase (G-6-Pase), a multifunctional enzyme[2], is the only principle enzyme of sugar metabolism associated with endoplasmic reticulum and is mainly found in liver, kidney and small intestine. At the intestinal level, G-6-Pase activity appears at the end of gestation[3]. After birth the activity increases during the 1st week, remains stable during the 2nd week and decreases to adult level in all intestinal segments during the 3rd week[4]. We have shown that its ultrastructural localization in the enterocytes is similar during the postnatal development[5] and in adult mouse[6]. The decrease of G-6-Pase activity observed during the 3rd postnatal week occurs when important enzymatic changes are taking place at the brush border level; sucrase activity appears, and the other brush border hydrolytic activities such as trehalase, glucoamylase, alkaline phosphatase and leucylnaphthylamidase increase to reach adult levels[4,7,8]. Since the enzymatic modifications occurring at the brush border membrane level are under control of glucocorticoids, thyroxine and insulin[7-13], we questioned in the present investigation whether the normal development of intestinal G-6-Pase is also controlled by these hormones.

Materials and methods. At 8 days of age, Swiss ICR mice received 1 injection per day during 3 days of the following hormones: 1. cortisone acetate suspension (Merk, Sharp & Dohme) diluted in saline, injected i.p. at a dosage of 25 µg/g b.wt/day; 2. DL-thyroxine (Sigma) dissolved in 0.005 N NaOH injected i.p. at a dosage of 1 µg/g b.wt/day and, 3. NPH insulin suspension (Connaught Laboratories Ltd., Ontario) diluted in saline injected s.c. at a dosage of 12.5 mU/g b.wt/day. Control animals were injected with equivalent volumes of saline. At the end of the experimental period, the intestines were removed immediately following decapitation, measured and cut into 3 equal parts. Each intestinal segment was weighed and homogenized in 99 vol. of ice-cold redistilled water. The phosphohydrolase activity of G-6-Pase was assayed as previously described[4,6] and the proteins were determined according to Lowry et al.[14]. The G-6-Pase activity was expressed as µmoles of phosphorus liberated per min · g of protein. Differences between the experimental groups were analyzed using Student's t-test.

Results and discussion. Daily administration of cortisone, thyroxine and insulin during 3 days at dosages known to induce precocious enzymatic modifications at the brush border membrane level of suckling mice and rats[7,13] does not provoke a premature decrease of G-6-Pase activity. Indeed, as shown in the table, G-6-Pase activity remains unchanged or even increases slightly following the different hormonal treatments. One has to remember that during the suckling period this activity is 4 times the adult level[4]. The specific metabolic functions of intestinal G-6-Pase have not been clearly defined. Even though the intestinal mucosa is not considered to be a gluconeogenic tissue, the possibility that it might be an important source of glucose production in certain circumstances has not been excluded, as reported for starved guinea-pigs[15]. The higher-than-adult values reported for the phosphohydrolase activity of G-6-Pase during the suckling period could be related to a gluconeogenic function of the mucosa during this period. The high G-6-Pase activity observed during the first 2 postnatal weeks has been associated, in part, with an important

Influence of cortisone, thyroxine and insulin on intestinal phosphohydrolase activity of glucose-6-phosphatase in suckling mice

	Number of animals	Small intestinal segments 1/3 P	1/3 M	1/3 D
Controls	6	40.3 ± 6.6	83.7 ± 5.8	47.8 ± 2.7
Cortisone	8	51.6 ± 4.3^{NS}	97.6 ± 5.2***	54.0 ± 2.9^{NS}
Thyroxine	9	61.8 ± 4.3*	83.9 ± 2.4^{NS}	56.9 ± 2.9**
Insulin	6	61.8 ± 3.6*	93.7 ± 2.2^{NS}	57.2 ± 2.2**

Glucose-6-phosphatase activity is reported for the proximal thirds (1/3 P), middle thirds (1/3 M) and distal thirds (1/3 D). Results are expressed as the mean of each group ± SEM. The hormonal treatments started at 8 days of age and the animals received 1 injection/day for 3 days of cortisone (25 µg/b b.wt), thyroxine (1 µg/g b.wt) and insulin (12.5 mU/g b.wt). The animals were sacrificed 24 h after the last injection. Levels of statistical significance of differences between control and experimental groups: * $p < 0.01$; ** $p < 0.025$; *** $p < 0.05$; NS, not significant.