Effect of cerebral cortex spreading depression on LH secretion

		No. of cases	No. of positive stimulations[a]	O.A.A.D. %	*P* value
(a)	Control (uniovariectomy) *bilateral*	12	—	-1 ± 1.9[b]	—
(b)	Bone removed	12	1	3.2 ± 2.5	vs. a. n.s.
(c)	Bone + dura removed	11	1	6.1 ± 1.2	vs. a. n.s.
(d)	(c) + local KCl solution	12	10	18.6 ± 2.0	vs. a. <0.001 vs. c. <0.001
	unilateral				
(e)	Bone + dura removed	12	2	5.1 ± 2.2	vs. a. n.s.
(f)	(e) + local KCl solution	19	5	8.1 ± 1.8	vs. a. < 0.01

[a] O.A.A.D. higher than mean control + 2 standard deviations. [b] Mean and standard error of mean. n.s. Not significant.

ovary as compared to the first was taken as an index of endogenous liberation of LH. Ovarian ascorbic acid depletion (O.A.A.D.) has been reported[2,3] as a very sensitive and specific test for LH.

As has been shown[4], spreading depression (S.D.) can easily be elicited in rats' cerebral cortex by local application of 25% KCl. This technique was used in the present experiment. Cotton soaked with 25% KCl was applied to the cerebral cortex after a window of 0.5 cm diameter was opened in the skull in the occipital area and the dura carefully removed under microscopic control in order to avoid cortical injury. Groups of animals were prepared in which only bone, or bone plus dura, were removed without application of KCl. These operations were performed immediately after the removal of the first ovary.

As can be seen in the Table, removal of one ovary does not induce O.A.A.D. In contrast to this, a highly significant O.A.A.D. takes place when 25% KCl is applied bilaterally to the cortex. Also unilateral application of KCl has induced a significant LH secretion. No effect was obtained by removing the bone alone or together with the dura.

As has been shown[4], S.D. not only produces a severe impairment of electrical spontaneous activity of the cortex but can also suppress its functional activity. In that way this technique can be used as a means to induce functional ablation of the neocortex.

The results obtained in the present work show that cortical S.D. evoked by local application of KCl induces a liberation of LH. This fact is an evidence that the neocortex activity is involved in the mechanisms of secretion of this hormone, probably through a modification of hypothalamic activity. BURES[4] has also reported, under the same circumstances, symptoms of hypothalamic involvement such as thermoregulation disturbances, water retention and hypoglycemia. The fact that stressful stimuli do not influence LH liberation[2] renders this phenomenon more evident.

In order to explain these results, we have to postulate that in normal conditions the cerebral cortex has an inhibitory influence on LH secretion and when its spontaneous activity is depressed either by its injury, as in the case of the needle, or by local application of 25% KCl, the inhibition is removed and release of the hormone takes place.

Résumé. La «spreading depression» du cortex cérébral provoquée chez les rats par l'application locale d'une solution de KCl à 25% déclenche une sécrétion d'hormone lutéinisante mesurée par la déplétion de l'acide ascorbique ovarien chez des animaux pseudogravides.

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Presynaptic Effect of the Neuro-Muscular Transmitter

At the neuro-muscular (n-m) junction a presynaptic effect of acetylcholine (ACh), and of certain compounds related to this drug, has been indicated by the drug conditioned antidromic nerve activity deseribed by MASLAND and WIGTON[1], FENG and LI[2], RIKER et al. (e.g. FUJIMORI et al.[3], RIKER et al.[4]), WERNER[5] and others, and discussed by KOELLE[6]. Since the postsynaptic transmitter effect may presumably influence the presynaptic events, it is of importance for the study of the latter to establish conditions which exclude the post-synaptic and maintain the presynaptic effect of the transmitter.

The following is a preliminary report on an attempt to exclude the postsynaptic response by cutting the muscle fibres transversally on either side of the endplate region, leaving the muscle fibres to depolarization by the de-

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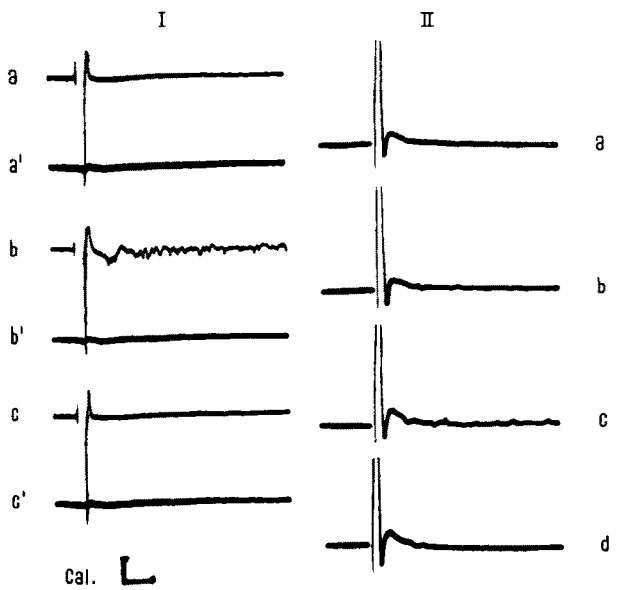
[6] G. B. KOELLE, Nature *190*, 208 (1961); J. Pharm. Pharmacol. *14*, 65 (1962).

marcation currents. A detailed description will be given later in the complete publication of the experiments. Isolated rat phrenic nerve-diaphragm preparations were used. Nerve action potentials and endplate potentials were recorded externally by means of platinum and chlorinated silver electrodes respectively. Resting potentials of muscle fibres were recorded intracellularly by means of glass microelectrodes filled with 3 molar KCl solution and having an impedance of 10–20 MΩ. The cut muscle fibres usually had a length of 4–5 mm.

The resting potential of the muscle fibres before cutting was found to be fairly stable, between 70 and 75 mV. No antidromic activity was recorded on supramaximal stimulation of the nerve (30/min). Addition of prostigmine or diisopropylfluorophosphate (DFP) caused the usual repetitive firing in the muscle and back firing in the nerve following orthodromic nerve volleys.

The cutting caused a fall of the muscle resting potential to about 30–35 mV within a few minutes, measured midway between the cut surfaces. Further decline took place slowly, the resting potential ending up at roughly 15 mV after 1 to 2 h. On nerve stimulation, no electrical muscle activity was recorded under these conditions except for a small and fading endplate potential being seen up to about half an hour after cutting. A short interval after addition of prostigmine (1 μg/ml of bath fluid) the orthodromic spikes were regularly followed by a burst of antidromic nerve action potentials lasting up to 40–50 msec (see the Figure). There was no activity recorded from the muscle and the resting potential in most muscle fibres was about 20 mV. Prostigmine had a more pronounced effect than DFP (2 μg/ml). Addition of ACh iodide (0.6 μg/ml) to a DFP-treated preparation caused, however, a significant increase in the antidromic firing following the orthodromic nerve volleys. ACh iodide in a concentration of 15 μg/ml extinguished the antidromic nerve potentials within 1 or 2 min. This also occurred when the back firing was due to prostigmine. *d*-Tubocurarine chloride (2.5 μg/ml) had a similar effect.



Drug conditioned antidromic activity in the rat phrenic nerve after injury depolarization of the diaphragm. Extracellular recording from nerve and muscle. Supramaximal indirect stimulation at 30/min. Pulse width, 0.05 msec. Negativity downwards in the left column, upwards in the right. The tracings a', b', and c' (left column) are from the muscle, the others from the nerve. Orthodromic spike far left in each nerve record. I, a, and a', 20 min after cutting of the muscle fibres, addition of prostigmine bromide, 1 μg/ml. b and b', 2 min after prostigmine addition. The orthodromic nerve spike is followed by antidromic nerve activity. Addition of *d*-tubocurarine chloride (*d*-Tc), 2,5 μg/ml. c and c', 2 min after *d*-Tc addition. Antidromic activity stopped. II, a, similar to I, a. DFP, 2 μg/ml added. b, 10 min after DFP addition. Slight antidromic firing. ACh iodide, 0.6 μg/ml, added. c, $1^1/_2$ min after ACh addition. Antidromic firing increased. Addition of ACh, 15 μg/ml. d, 2 min after c, antidromic activity almost stopped. Time calibration, 2 msec. Amplitude calibration, 0.5 mV and 0.2 mV for nerve and muscle records respectively. For further details see text.

The antidromic spikes have been claimed to be generated by either of two mechanisms, viz. an electrical stimulation of the nerve terminals from the muscle fibres (ephaptic transmission) during the muscle activity, particularly during the repetitive firing in the muscle, or a presynaptic effect of the transmitter. A combination of both these mechanisms is also a possibility to be considered.

Though no activity was recorded from the cut muscle fibres in the present experiments, the postsynaptic phenomena of demarcation (e.g. injury current and alteration of the ion environment) could presumably influence the presynaptic terminals. Nerve terminals which are cut together with the muscle fibres will certainly develop their own demarcation currents. Thus it cannot be excluded that phenomena other than the transmitter effect may play an important part at the nerve endings under the experimental conditions described. The antidromic activity in the stimulated motor nerve following addition of a cholinesterase-inhibitor to the bath fluid, when the cut muscle was depolarized to about 20 mV and the last trace of a muscle response, not to say repetitive firing of the muscle, was lost, seems to indicate a presynaptic effect of the transmitter. The effect of ACh on the back firing due to DFP, also indicates an ACh sensitivity of the presynaptic terminals. On this assumption it is not surprising that the backfiring was blocked by excess of ACh and by *d*-tubocurarine in accordance with the effects of these drugs on the postsynaptic receptors. The transmitter probably contributes to the generation at the nerve endings of a graded local response, which outlasts the refractory period of the adjacent 'all or nothing' responding nerve membrane, and sets up antidromic repetitive activity in the nerve. These results seem to support the hypothesis put forward by KOELLE[6].

Zusammenfassung. Nach transversaler Durchschneidung der Muskelfasern des isolierten Rattenzwerchfelles auf jeder Seite der Endplattenregion wurde die elektrische Aktivität im stimulierten Nervus phrenicus nach Zusatz von Prostigmin, DFP und zum Teil auch Acetylcholin studiert. Die orthodromen Nervenimpulse wurden von antidromer Aktivität im Nerv gefolgt, auch dann, wenn durch Demarkationsvorgänge das Ruhepotential des Muskels bis auf 20 mV heruntergebracht und die elektrische Aktivität im Muskel gelöscht war. Dies scheint für eine praesynaptische Wirkung des Acetylcholins zu sprechen.

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