Effect of Calcium Ions on the Release of Acetylcholine from the Cerebral Cortex

THE presence of calcium ions (Ca^{2+}) in the external medium is essential for the release of acetylcholine (ACh) at peripheral synapses (superior cervical ganglion, neuromuscular junction)[1-6]. Evidence now suggests that ACh is a transmitter in the cerebral cortex, and several investigators have shown that this substance is continuously released from the cerebral cortex in amounts which can be directly related to the level of cortical activity[7-12]. This communication describes how changes in local Ca^{2+} affect the spontaneous and evoked release of ACh from the cat cerebral cortex.

Table 1. MEAN RATE OF ACETYLCHOLINE RELEASE (ng/min/cm²) FROM UNSTIMULATED CEREBRAL CORTEX INTO RINGER–LOCKE SOLUTION CONTAINING DIFFERENT CONCENTRATIONS OF Ca^{2+}

Region	No. of cats	Rate in 2 mmolar	Rate in 1 mmolar	Rate in 0·2 mmolar	Rate in 0 mmolar	P
Sensory-motor and parietal cortex	12	0·74 ± 0·09 (*S.E.*)	—	—	0·49 ± 0·06	< 0·05
	2	0·90 ± 0·20	0·81 ± 0·19	—	—	*
Sensory-motor cortex	4	0·48 ± 0·12	—	0·36 ± 0·07	—	*

* These data are of a preliminary nature, but they suggest that there is no effect of low-Ca^{2+} solution (0·2 mmolar and 1·0 mmolar) on the rate of release of ACh as compared with the control values.

Experiments were performed on cats anaesthetized with a mixture of diallyl–barbituric acid and urethane ('Dial', Ciba, Ltd.) given intraperitoneally (0·7–0·8 ml./kg). ACh was collected from the exposed surface (1 cm²) of the pericruciate and suprasylvian cortex, using the 'Perspex' chamber technique described before[7]. The chambers were filled with 1 ml. of Ringer–Locke solution containing eserine sulphate (1×10^{-4} g/ml., British Drug Houses) and aerated with 5 per cent carbon dioxide in oxygen. The Ringer–Locke solution used had the following ionic composition: 137·0 mmolar sodium chloride; 2·5 mmolar potassium chloride; 2·0 mmolar calcium chloride; 1·0 mmolar magnesium chloride; 1 mmolar sodium phosphate; 12·0 mmolar sodium bicarbonate; and 11·0 mmolar glucose.

The concentration of Ca^{2+} was varied in different experiments (Ca^{2+}-free, 0·2, 1·0 and 2·0 mmolar) by omitting calcium chloride; the other ions and the concentration of eserine remained constant. The small changes in osmolarity were not corrected for. The stimulating pulses were usually rectangular pulses lasting 1 msec, delivered at a frequency of 0·5/sec to the contralateral forepaw, through a radio-frequency isolation unit of the Grass '*S8*' stimulator. Efficiency of stimulation was checked by recording the evoked potential with a cathode-ray oscilloscope. ACh was sampled from the surface of the cerebral cortex unilaterally for between fifteen and eighteen collection periods, each of 15 min duration. The usual procedure was as follows: two resting samples alternated with a stimulated one, first with normal (2·0 mmolar Ca^{2+}) Ringer–Locke and then the sequence was repeated with low-Ca^{2+} or Ca^{2+}-free solution. In this way, we were able to detect a significant change in the release of ACh, which was reproducible. Immediately after removal, samples were frozen at −15° C and stored at that temperature.

Bio-assays were performed within 48 h of collection on the dorsal muscle of the leech (*Hirudo medicinalis*). As a control, muscle was soaked for 10 min in a solution of *d*-tubocurarine chloride (10^{-5} g/ml., California Biochemical Corporation). This procedure abolished the response to standard solutions of ACh, as well as to the active principle present in the samples. The results were analysed statistically by using Student's t test.

When the normal Ringer–Locke solution was changed for one containing no calcium chloride, there was a significant fall (of 34 per cent on average) in the amount of ACh collected from the surface of the cerebral cortex in the absence of peripheral stimulation (Table 1). The depressant effect of the lack of Ca^{2+} was readily reversed by restoring Ca^{2+} to the collection fluid. It is interesting that the action of Ca^{2+} is exerted only on a portion of the spontaneous release of ACh, while the remaining fraction was unaffected by the absence of calcium. This result is qualitatively in agreement with the data obtained at mammalian motor nerve terminals[4-6].

Mitchell[8] has reported that electrical stimulation of a peripheral sensory nerve produced an increase in the rate at which ACh was liberated from the surface of the contralateral sensory cortex. Using this procedure we have found that with 0·2 mmolar Ca^{2+} and with Ca^{2+}-free solutions there is no increase in the output of ACh during peripheral stimulation (Table 2). These results are of interest, because in all synapses so far examined the release of the chemical transmitter by the nerve impulse requires the presence of Ca^{2+} in the external medium. It seems that the cerebral cortex is not an exception to this. These observations also provide supporting evidence for the involvement of ACh as a synaptic transmitter in the cerebral cortex.

Table 2. MEAN RATE OF SPONTANEOUS AND EVOKED ACETYLCHOLINE RELEASE (ng/min/cm²) FROM CEREBRAL CORTEX INTO RINGER–LOCKE SOLUTION CONTAINING DIFFERENT CONCENTRATIONS OF Ca^{2+}

Region	No. of cats	[Ca^{2+}]	Resting rate	Rate on contralateral forepaw stimulation	P
Sensory-motor cortex	8	2 mmolar	0·54 ± 0·09 (*S.E.*)	0·81 ± 0·09	< 0·05
	8	0·2 ± 0 mmolar	0·39 ± 0·05	0·42 ± 0·05	Not significant

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M. RANDIĆ
A. PADJEN

Biology Division, Institute "Rudjer Bošković",
and Department of Pharmacology,
Medical Faculty,
University of Zagreb.

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