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THE EFFECT OF SODIUM ON DEPOLARIZATION-INDUCED CALCIUM UPTAKE AND ACETYLCHOLINE RELEASE BY SYNAPTOSOMES

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The influence of external sodium concentration on potassium (depolarizing agent)-stimulated calcium uptake and Ca^+-dependent acetylcholine release by rat cerebral cortex synaptosomes has been studied. It was found that increased sodium concentration decreases both the Ca^{2+} uptake and the acetylcholine release, whereas a low external sodium concentration is stimulatory.

Potential-dependent changes in permeability to sodium and potassium ions enable a nerve to conduct impulses which, arriving at the nerve terminal, elicit an influx of Ca^{2+} and subsequent release of neurotransmitter. However, the mechanism by which calcium shifts occur upon depolarization is not well understood. According to current views [1], two mechanisms of calcium flux in rat brain presynaptic terminals, operating parallel but independently, may be involved: depolarization-induced calcium uptake and Na^+-Ca^{2+} exchange. Depolarization-stimulated calcium uptake and Ca^{2+}-dependent transmitter release by rat-brain synaptosomes is well established [2], but the contribution of the Na^+-Ca^{2+} exchange mechanism to calcium uptake by synaptosomes is not clear [3]. It was found [4] that the increase in fluorescence (which is directly proportional to membrane potential) obtained by increasing the external K^+ concentration $[K]_o$ does not depend on $[Na]_o$, and that low $[Na]_o$ inreases Ca^{2+} uptake by synaptosomes [5] as well as raising $[Na]_i$ [1]. Finally, it was shown that increased $[Na]_o$ antagonizes Ca^{2+} uptake [1]. This and preceding statements suggest that, at least to certain degree, the Ca^{2+} uptake can be regulated by an agent which does not influence membrane potential. Since, to our knowledge, there are no data concerning the significance of the correlation of the $[Na]_o$ effect on calcium uptake and depolarization-induced acetylcholine release, the present study was undertaken in order to find out whether changes in external sodium concentration affect the release of this transmitter by rat cerebral cortex synaptosomes.

Synaptosomes prepared by the method of Cotman and Matheus [6] or crude mitochondrial (P_2) preparation [7] were suspended in incubation medium A containing (in mM): 142 NaCl, 4 KCl, 2 $MgCl_2$, 10 glucose, 25 Tris-HCl (pH 7.4) and 2 $CaCl_2$ unless otherwise indicated. When the K^+ concentration was raised, the Na^+ concentration was decreased by an equivalent amount, except in experiments where the effects of $[Na]_o$ were examined. In these cases synaptosomes were transferred to medium A with low $[Na^+]$ or to medium A. Acetylcholine release started immediately upon addition of depolarization agent (KCl) to a final concentration of 30 mM. Uptake of $^{45}Ca^{2+}$ was followed in synaptosomes suspended in calcium-free medium A and medium A with low NaCl concentration. The uptake was also quickly started, after synaptosomes had been transferred to the appropriate medium, by simul-

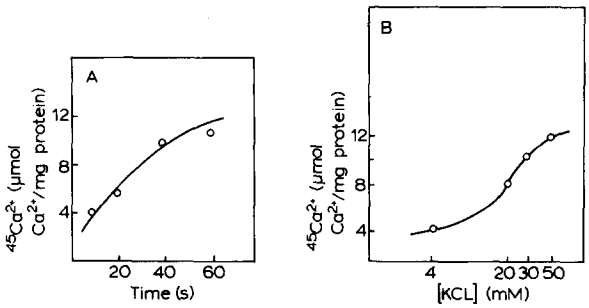

Fig. 1. Kinetics of K^+-stimulated calcium uptake in the presence of 30 mM KCl (A) and as a function of KCl concentration (B). Experiments were carried out using synaptosomes suspended in medium A lacking $CaCl_2$ and in which Na^+ was decreased by an equivalent amount of K^+. Reaction was started by addition of conc. KCl solution and 0.5 μmol of $^{45}Ca^{2+}$ (1 μmol/1 μCi; 1 Ci=37 GBq). Synaptosome concentration was 100 μg protein in a final volume of 200 μl.

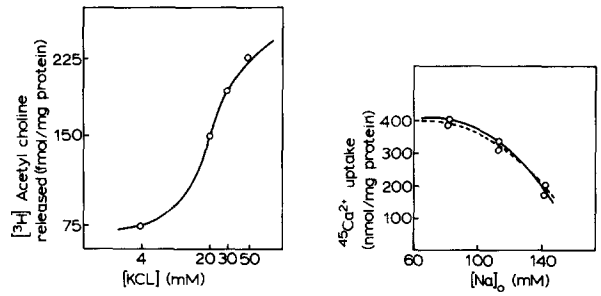

Fig. 2. (Left.) Acetyl[^{3}H]choline release as a function of KCl concentration. The synaptosomes suspended in medium A (1 mg/ml) were incubated with 2 μCi [*methyl*-^{3}H]choline chloride (89 Ci/mmol) for 30 min at 37°C. Loaded synaptosomes were washed twice and suspended in incubation medium containing 0.05 mM eserine, the sum of NaCl+KCl being 142 mM. Release was followed for 60 s.

Fig. 3. (Right.) Effect of $[Na^+]$ on the rate of $^{45}Ca^{2+}$ uptake. Synaptosome $^{45}Ca^{2+}$ uptake was followed for 30 s after KCl and $^{45}Ca^{2+}$ had been added. The KCl concentration was 30 mM, NaCl as indicated and 100 μg of synaptosome proteins were present in final volume of 200 μl. Reduced NaCl concentration was replaced by sucrose (O------O). The tonicity was not compensated (O——— O).

taneous additions of $^{45}Ca^{2+}$ and KCl. The acetyl[^{3}H]choline release from prelabeled synaptosomes, or P_2 fraction,with [^{3}H]choline was studied by the method of McClure et al. [8] which enables selective precipitation of acetylcholine.

The time course of $^{45}Ca^{2+}$ influx is presented in Fig. 1A. The elevation of K^+ concentration in the medium A results in the stimulation of the rate of $^{45}Ca^{2+}$ uptake by synaptosomes. The effect continues up to about 50 mM KCl (Fig. 1B). An increase in external K^+ concentration stimulates synaptosome acetyl[^{3}H]choline release which is also dependent upon KCl concentration in the medium (Fig. 2). The ability of rat-brain synaptosomes to incorporate $^{45}Ca^{2+}$ and release acetyl[^{3}H]choline after transfer to medium A of various NaCl concentrations is shown in Table I and Fig. 3. Results obtained for $^{45}Ca^{2+}$ uptake in response to low and high $[Na]_o$ are in agreement with results already reported [1,5]. Our studies were extended to the sodium effects upon acetylcholine release and show that reduced $[Na]_o$ stimulates and high $[Na]_o$ inhibits acetyl-[^{3}H]choline release by synaptosomes. The facility of Ca^{2+} uptake by low $[Na^+]$ was also reflected in transmitter release under conditions where Ca^{2+} was omitted from medium A, signifying that calcium had not been completely removed.

Our findings suggest that changes in $[Na^+]$ in the synaptosome incubation medium may in-

TABLE I

EFFECTS OF CATION CONCENTRATION ON THE RATE OF ACETYL[^{3}H]CHOLINE RELEASE BY SYNAPTOSOMES

Synaptosomes suspended in incubation medium (mg/ml) were labeled for 30 min with [^{3}H]choline. After washing, synaptosomes were suspended in media with appropriate NaCl concentrations. Reaction was started by addition of aliquots of conc. KCl and release was followed for 60 s.

Incubation medium (cation concentrations; mM)		Acetyl[^{3}H]choline release (fmol/mg protein)
NaCl	KCl	
142		
	30	132
	4	31
	30, Ca^{2+}-free	51
112		
	30	184
	4	37
	30, Ca^{2+}-free	51
82		
	30	217
	4	48
	30, Ca^{2+}-free	112

fluence the calcium-transport mechanism differently from the K^+-stimulated calcium uptake. The foregoing considerations support the idea that an Na^+-Ca^{2+} exchange mechanism may be involved in Ca^{2+} uptakeby rat-brain synaptosomes.

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References

1 Blaustein, M.P. and Oborn, C.J. (1975) J. Physiol. 247, 657–686

2 Blaustein, M.P., Johnson, E.M. and Neodlemann, P. (1972) Proc. Natl. Acad. Sci. U.S.A. 69, 2237–2240

3 Åkerman, K.E.O. and Nicholls, D.G. (1981) Eur. J. Biochem. 117, 491–497

4 Blaustein, M.P. and Goldring, J.M. (1975) J. Physiol. 247, 589–615

5 Swanson, P.D., Anderson, L. and Stahl, W.L. (1974) Biochim. Biophys. Acta 356, 174–183

6 Cotman, C.W. and Matheus, D.A. (1971) Biochim. Biophys. Acta 249, 380–394

7 Gray, E.G. and Whittaker, V.P. (1962) J. Anat. 96, 79–88

8 McClure, W.O., Abbott, B.C., Baxtor, D.E., Hsiao, T.H., Satin, L.S., Siger, A. and Yoshino, J.E. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 1219–1223