BBA 71023

Stimulatory effect of Na^+ and ATP on the release of acetylcholine from synaptic vesicles

Evidence that acetylcholine is an important neurotransmitter not only in the peripheral but also in the central nervous system has been accumulated by many investigators. It is also known that a large part of the acetylcholine in the brain is localized in the synaptic vesicle fraction[1,2]. Accordingly, in order to study the mechanism of release of acetylcholine, the effects of ions and various nucleotides on its release from the vesicle fraction of rat brain were examined.

The homogenate of rat cerebrum was fractionated as described by WHITTAKER, MICHAELSON AND KIRKLAND[2] and GRAY AND WHITTAKER[3] with some modifications. Acetylcholine was determined by the method of CHANG AND GADDUM[4].

The release of acetylcholine from the crude synaptic vesicle fraction was dependent upon the temperature of the incubation medium, as shown in Fig. 1. 50% of

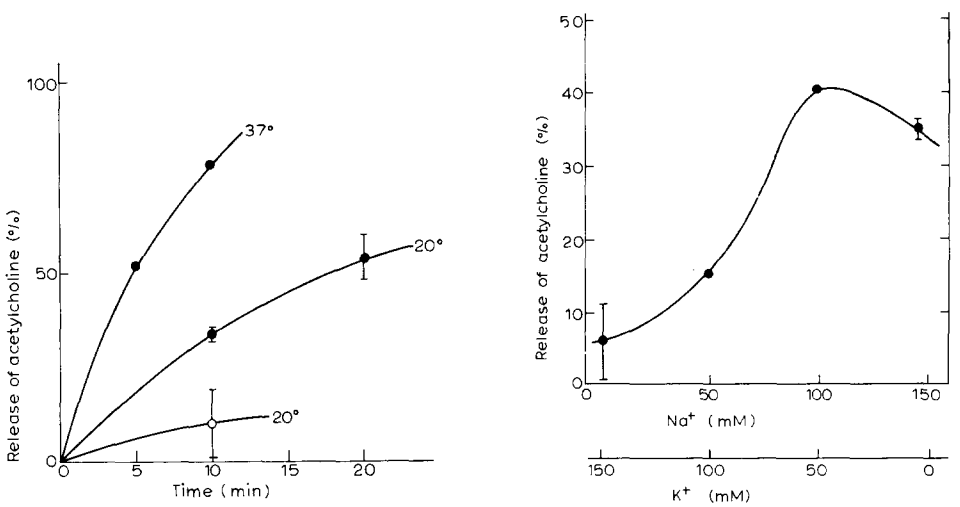

Fig. 1. Effect of temperature of the incubation medium on release of acetylcholine from synaptic vesicles. The cerebrums of Sprague–Dawley rats were homogenized in 0.32 M sucrose, pH 7.4 (10 ml/g of original tissue), with a Potter–Elvehjem glass-teflon homogenizer rotating at 400 to 600 rev./min. The homogenate was centrifuged at 900 × *g* for 10 min. The resulting supernatant was centrifuged at 17500 × *g* for 60 min to obtain the mitochondrial fraction including the synaptosome. The precipitate was suspended in distilled water (4 ml/g of original tissue) and stood for 5 min at 0° to disrupt the particles. The suspension was centrifuged at 115000 × *g* for 20 min. Then, the supernatant fluid was centrifuged at 105000 × *g* for 60 min. The precipitate, suspended in an isotonic medium, was used as the crude synaptic-vesicle fraction. 3 ml of incubation medium containing the synaptic-vesicle fraction (total acetylcholine, 1.80 mμmoles), 0.05 mM eserine sulfate and the following ions: ●—●, 150 mM NaCl, 2 mM KCl; ○—○, 150 mM KCl, pH 6.9. After incubation, released and particle-bound acetylcholine were separated by centrifugation at 105000 × *g* for 30 min at 4°. The particle-bound acetylcholine was determined by heating the sample at 100° for 10 min at pH 4.0. The acetylcholine released is expressed as a percentage of the total acetylcholine in the synaptic-vesicle fraction. Each point represents the average of the values of 4 experiments and the vertical lines indicate S.E.

Fig. 2. Effect of concentrations of Na^+ and K^+ on release of acetylcholine from synaptic vesicles. Incubation medium (pH 6.9) contained synaptic-vesicle fraction (total acetylcholine, 1.17 mμmoles), 0.05 mM eserine sulfate and NaCl and KCl as indicated in the figure. The incubation was at 20° for 10 min. For explanation of symbols, see Fig. 1.

the total acetylcholine was released from the synaptic vesicles in about 20 min at 20° in isotonic sodium medium (150 mM NaCl, 2 mM KCl, 0.05 mM eserine, pH 6.9). In isotonic K^+ medium (150 mM KCl, 0.05 mM eserine, pH 6.9), however, the release of acetylcholine was slower, being about one-third that in isotonic sodium medium on incubation at 20° for 10 min. Fig. 2 shows how the release of acetylcholine from the synaptic vesicles varies with the concentrations of NaCl and KCl in the incubation medium. The release of acetylcholine was stimulated by high Na^+ and low K^+ concentrations. On arrival of an impulse at the nerve endings, there is an increased influx of Na^+ into the cell and an increased efflux of K^+. As a result, in the intracellular ionic environment surrounding the synaptic vesicles, the Na^+ concentration increases and the K^+ concentration decreases. The coupling between the nerve impulse and the release of acetylcholine may be explained by the stimulatory effect of Na^+ on the release of acetylcholine from the synaptic vesicles.

The release of acetylcholine from synaptic vesicles was also stimulated by the addition of ATP to the incubation medium. As shown in Fig. 3A, the release of acetylcholine into the medium on addition of 2.5 mM ATP *plus* Mg^{2+} was more than twice that of the control. At concentrations of ATP above 2.5 mM, there was no further increase in the stimulatory effect. The addition of ATP alone in the absence of Mg^{2+} also had a marked effect. This seems to be of interest and is in contrast to the findings

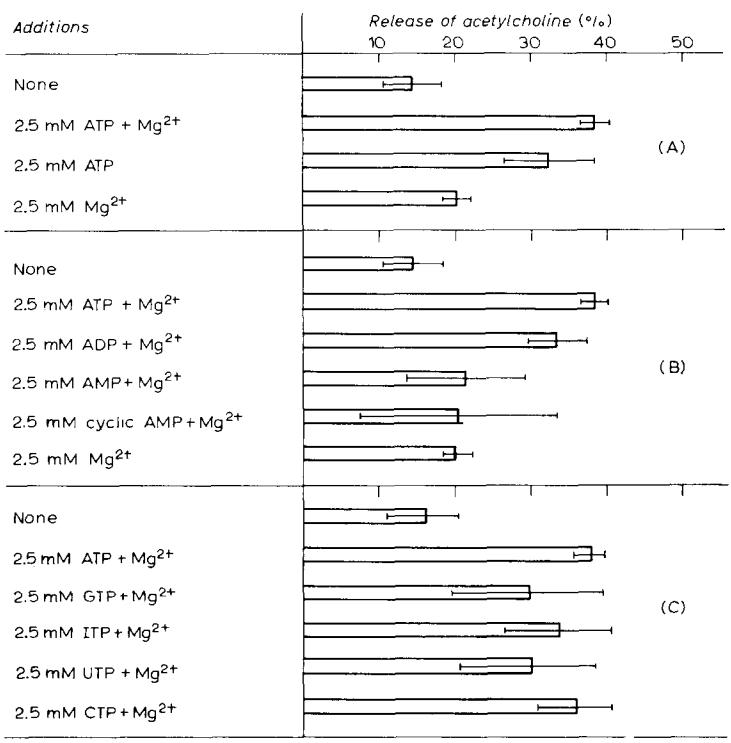

Fig. 3. Effect of ATP and other nucleotides on the release of acetylcholine from synaptic vesicles. Incubation medium contained the synaptic-vesicle fraction (total acetylcholine, 1.65–1.89 mμmoles), 5 mM sodium phosphate buffer (pH 7.4), 0.05 mM eserine sulfate, 0.5 mM GEDTA, 150 mM KCl and the additions indicated in the figure. The incubation was at 20° for 10 min. The bars represent the means of 4 experiments. The horizontal lines indicate S.E.

of others of a Mg^{2+}-dependent ATPase in the vesicles, as discussed later. The effects of adenine nucleotides other than ATP are summarized in Fig. 3B. ADP had less effect than ATP. AMP and cyclic 3′,5′-AMP had little or no effect. Addition of nucleoside triphosphates other than ATP, such as ITP and CTP, stimulated the release of acetylcholine nearly as much as ATP, while GTP and UTP had a lesser effect (Fig. 3C).

The existence of a Mg^{2+}-dependent ATPase in the synaptic vesicles of brain has been reported[5–7]. However, our results suggest that there is no direct relation between the effect of ATP and the ATPase. ATP may affect the membrane structure of the synaptic vesicles. However, it has been reported that the release of biologically active substances other than acetylcholine is also stimulated by the addition of ATP *plus* Mg^{2+} to the incubation medium. For example, the release of catecholamines from isolated granulated vesicles of adrenal medulla[8] and that of amylase from isolated zymogen granules of salivary gland[9] are stimulated by ATP *plus* Mg^{2+}. From these reports and our findings, it seems likely that ATP acts on many kinds of intracellular structures and stimulates the release of various, stored, biologically active substances by different mechanisms. But the mechanism of action of ATP on the membrane and the role of ATP as an intracellular regulator remain unknown.

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1 E. DE ROBERTIS, G. R. DE L. ARNAIZ, L. SALGANICOFF, A. P. DE IRALDI AND L. M. ZIEHER, *J. Neurochem.*, 10 (1963) 255.
2 V. P. WHITTAKER, I. A. MICHAELSON AND R. J. A. KIRKLAND, *Biochem. J.*, 90 (1964) 293.
3 E. G. GRAY AND V. P. WHITTAKER, *J. Anat., Lond.*, 96 (1962) 79.
4 H. C. CHANG AND J. H. GADDUM, *J. Physiol.*, 79 (1933) 255.
5 R. J. A. HOSIE, *Biochem. J.*, 96 (1965) 404.
6 M. GERMAIN AND P. PROULX, *Biochem. Pharmacol.*, 14 (1965) 1815.
7 K. KADOTA, S. MORI AND R. IMAIZUMI, *J. Biochem. Tokyo*, 61 (1967) 424.
8 M. OKA, T. OHUCHI, H. YOSHIDA AND R. IMAIZUMI, *Biochim. Biophys. Acta*, 97 (1965) 170.
9 H. YOSHIDA, H. ISHIDA, N. MIKI AND I. YAMAMOTO, *Biochim. Biophys. Acta*, 158 (1968) 487.

Received March 18th, 1968