SUBCELLULAR DISTRIBUTION OF ASCORBIC ACID IN RAT BRAIN

Che-Hui KUO, Norifumi YONEHARA, Fumiaki HATA
and Hiroshi YOSHIDA

*Department of Pharmacology I, Osaka University, School of Medicine,
Kita-ku, Osaka 530, Japan*

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It is well documented that influx of Ca ion to the nerve terminal induces a release of acetylcholine (ACh), the nerve transmitter (1). One of the sites of action of Ca^{++} in the nerve terminal is considered to be on synaptic vesicles which store ACh. Accordingly, we isolated the synaptic vesicles from the rat brain and conditions which would induce a release of ACh from the vesicles responding to low concentration of Ca^{++} were examined. As we have already reported, Ca^{++} dependent (10^{-6} M) ACh release from the synaptic vesicles was enhanced by the addition of sap (synaptosomal cytoplasm) in the presence of ATP and Mg^{++} (2-3). The factor in the sap was then purified by column chromatography and was identified to be ascorbic acid, with UV-spectrum in various pH conditions, and cellulose thin layer chromatography. During the above experiments, we observed that the releasing effects of sap were more remarkable than that of the cytoplasm in the cell body. Distribution of ascorbic acid in the subcellular fractions of rat brain was examined herein.

Subcellular fractionation was carried out by method of Whittaker et al. (4) with a slight modification as follows: whole brains of adult healthy S.D. rats were homogenized with 5 vol of 0.32 M sucrose and the soluble fraction (S_1) was obtained by centrifugation at $1,000 \times g$ for 10 min. Then S_1 fraction was centrifuged at $12,000 g$ for 40 min to obtain particle (P_2) and soluble (S_2) fractions. After hypotonic treatment of P_2 fraction with water (4 ml/g tissue), centrifuged at $100,000 \times g$ was carried out for 40 min and soluble (P_2s) and particle (P_2p) fractions were obtained. Microsome and S_3 fractions were obtained by centrifugation of S_2 at $100,000 \times g$ for 40 min. Content of ascorbic acid in each fraction was determined with 2,4-dinitrophenylhydrazine by method of Lowry et al. (5) and protein were measured according to method of Lowry et al. (6).

Content of ascorbic acid in rat brain homogenate was $357.3 \pm 21.9 \mu\text{g/g}$ tissue and this value is in agreement with the results of Subramanian (7). Table 1 shows the subcellular distribution of protein and ascorbic acid per g wet weight of the rat brain. As shown in Table 1, fraction P_2s , synaptosomal cytoplasm, exhibited the highest specific content of ascorbic acid ($\mu\text{g/mg}$ protein) among the subfractions. S_3 had also high specific content, but significantly less than P_2s . Particles had little ascorbic acid.

Ascorbic acid is well known to be an anti-scurvy vitamin, but its physiological role in brain function is unknown. Ascorbic acid was found in the brain in the highest concentration, except for the adrenals (8), and it was reported that ascorbic acid was taken up by the brain

TABLE 1. Subcellular distribution of ascorbic acid in rat brain

	mg protein/g	μ g ascorbic acid/g	μ g ascorbic acid/mg prot
Homogenate	108.1 $\pm$ 16.6	357.3 $\pm$ 21.9	3.4 $\pm$ 0.6
P ₁	79.5 $\pm$ 13.6	190.6 $\pm$ 9.8	2.4 $\pm$ 0.3
S ₁	30.8 $\pm$ 3.2	150.4 $\pm$ 10.4	4.9 $\pm$ 0.2
P ₂	13.3 $\pm$ 1.1	32.1 $\pm$ 5.6	2.5 $\pm$ 0.4
S ₂	12.3 $\pm$ 3.2	116.4 $\pm$ 23.0	9.6 $\pm$ 0.9
P ₂ p	9.6 $\pm$ 1.0	2.5 $\pm$ 0.3	0.3 $\pm$ 0.1
P ₂ s	2.4 $\pm$ 0.4	36.7 $\pm$ 8.2	15.5 $\pm$ 1.9*
Microsome	1.8 $\pm$ 0.4	3.7 $\pm$ 1.7	2.0 $\pm$ 0.5
S ₃	9.9 $\pm$ 2.8	104.3 $\pm$ 19.9	10.8 $\pm$ 1.0*
**Recovery (%)	95.8 $\pm$ 6.2	94.6 $\pm$ 2.2	

The results are the mean $\pm$ S.D. of 4 experiments. *Significantly different between contents of ascorbic acid in P₂s and S₃ fractions ($p < 0.025$). **P₁ + P₂p + P₂s + microsome + S₃

from plasma by a carrier mediated, energy-dependent and stereospecific transport system (9). Furthermore, other workers found that vitamin C participates in synthesis and storage of catecholamine (10–11), and in vitamin C deficient states, significant changes in the amount of catecholamines in the brain have also been reported (12). In our laboratory we found that the low concentration of L-ascorbic acid (5×10^{-6} M) induced a Ca^{++} -dependent (10^{-6} – 10^{-5} M) ACh release from the isolated synaptic vesicles in the presence of ATP and Mg^{++} . These results indicate that ascorbic acid may be associated with the function of the nervous system, particularly with dynamic states of transmitters in the nerve terminals. Under physiological conditions, concentration of ascorbic acid in the brain was found to be over 2 mM, such levels being sufficient for ACh release. Accordingly the ACh release from the vesicles is considered to be physiologically regulated by Ca^{++} .

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THE OXYTOCIC EFFECT OF CENTRALLY INJECTED PROSTAGLANDINS E_2 AND $F_{2\alpha}$

Seigo FUJIMOTO, Shiro HISADA and Hiromi TSUSHIMA

*Department of Pharmacology, Nagoya City University Medical School,
Kawasumi, Mizuho-ku, Nagoya 467, Japan*

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Centrally administered prostaglandins (PGs) released hypothalamic and pituitary hormones including antidiuretic hormone (ADH) in rats (1, 2). The present authors reported that PGE_2 and $PGF_{2\alpha}$, when injected into the lateral ventricle produced diuresis followed by antidiuresis in ethanol anaesthetized rats (3). Centrally injected PGA_2 also changed urine outflow in the rat (4). On the other hand, little attention has been paid to effect of centrally injected PGs on oxytocin release, although Prilusky and Deis (5) did suggest that i.p. administration of $PGF_{2\alpha}$ inhibited milk-ejection by a central block on oxytocin release in lactating rats and Cobo *et al.* (6) suggested that the milk-ejecting activity of i.v. administered $PGF_{2\alpha}$ in lactating women was mediated through the central release of oxytocin.

In the present study, an attempt was made to determine whether or not centrally administered PGE_2 and $PGF_{2\alpha}$ were oxytocic in rats. Virgin Wistar rats, weighing 200-250 g were used. The sexual stage of the rat was determined by taking a vaginal smear in the morning before the rat was selected for the experiment. Ethanol was used as the anaesthetic according to method of Krejčí *et al.* (7). Essentially, anaesthesia was induced by oral administration of 12% ethanol in a volume of 5 ml/100 g and maintained with i.v. infusion of 3% ethanol at a rate of 0.1 ml/min. The technique for recording the spontaneous motility of the uterus was as described elsewhere (8). Briefly, the horn of one uterus was cannulated with a polyethylene tubing filled with saline and the spontaneous motility of the uterus as reflected in the intrauterine pressure was recorded through a pressure transducer on a polygraph. A base line pressure of 30-40 mmH₂O was introduced into the uterine horn.

PGE_2 and $PGF_{2\alpha}$ (Ono Pharmaceutical Co., Ltd., Osaka) were injected into the lateral ventricle at a constant volume of 10 μ l. Both PGs were dissolved before use in small amounts of absolute ethanol and diluted by artificial cerebrospinal fluid (CSF). The composition (in mEq/l) of the CSF was as follows: Na⁺; 150; K⁺; 3; Ca⁺⁺; 2.3; Mg⁺⁺; 1.6; Cl⁻; 135; HCO₃⁻; 21; HPO₄⁻; 0.5. Ethanol concentration of 10 nmoles/10 μ l of PGE_2 and $PGF_{2\alpha}$ was 3%. Oxytocin (Atonin-O, Teikoku Hormone MFG Co., Ltd., Tokyo) was diluted with saline for i.v. administration in a volume of 0.1 ml. When 3% ethanol or CSF was