Effets de la pentagastrine et de l'histamine sur la teneur en acide ascorbique de la muqueuse de rat

Agent	Dose (mg/kg)	Délai (min)	Poids moyen des animaux (g)	Acide ascorbique de la muqueuse gastrique Témoin (µg/g)	Traités (µg/g)	Comparaison	Corrélation entre le pH et l'acide ascorbique
Pentagastrine	0,5	60	180	331 ± 39 (7)	289 ± 18,7 (8)	n.s.	n.s.
Pentagastrine	1,5	30	230	200 ± 9,7 (12)	138 ± 8,3 (12)	$p < 0,001$	$p < 0,02$
Histamine	30	60	231	288 ± 34 (6)	265 ± 48 (6)	n.s.	n.s.
Histamine	100	30	220	222 ± 8,0 (7)	194 ± 10 (8)	$p < 0,05$	n.s.
Histamine	100	60	220	203 ± 23 (8)	181 ± 15 (8)	n.s.	n.s.

Nombre de rats entre parenthèses; n.s., non significatif. $m \pm s_m$

à celle du pH gastrique par une corrélation significative. Nous retrouvons ici un phénomène identique à celui que nous avons déjà décrit pour le DG. L'injection d'HT provoque également une diminution de l'AA de la muqueuse mais à des doses élevées qui peuvent s'expliquer par la relative insensibilité de l'espèce et par la voie d'administration retenue[5]. Nous n'avons pas trouvé de modification du pH gastrique ni de corrélation entre l'AA et le pH de la muqueuse. L'étude du débit acide dans d'autres conditions expérimentales permettrait peut-être de retrouver cette corrélation.

En conclusion les stimulations hormonales (PG, HT) et nerveuses (par le vague après DG) provoquent dans nos conditions expérimentales une sécrétion gastrique acide et un abaissement de l'AA de la muqueuse. Associés à l'utilisation en thérapeutique antiulcéreuse de l'AA[6], ces faits mettent en évidence la nécessité d'une étude plus importante du rôle de l'AA dans la physiologie de la sécrétion gastrique.

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Nerve endings isolated from chick embryonic optic tectum. 1. Developmental aspects of intact synaptosomes

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Summary. Fractions enriched in nerve endings (synaptosomes) have been isolated from optic tectum of chick embryos at 16 and 18 days of incubation and of chicks immediately after hatching. Morphological aspects of nerve endings have been examined with special regard to the appearance of synaptic thickenings and synaptic vesicles.

When brain tissue is homogenized in iso-osmotic aqueous sucrose media under conditions of moderate shear force, artifacts named 'synaptosomes' are formed by the pinching-off and selfseeling of synaptic contacts between nerve cells[1]. Those particles seem to maintain the morphological features and the chemical composition of intact synaptic terminals, including elements necessary for transmission signals and maintenance of synaptic contacts. Since they can be separated from other subcellular particles in reasonable yield and with high purity, they have been utilized in a multitude of studies designate to illuminate many aspects of synaptic function. Until recently, neurochemists have expended much effort to purify synaptosomes and subsynaptosomal fractions from adult brain[2], but observations on nerve endings isolated from immature brain are rare[3], whereas the study of synaptic immature contacts could be a tool for clarifing many mechanisms which act in the formation of neural circuits. This paper describes some morphological aspects of fractions enriched in nerve endings (synaptosomes) isolated from the optic tectum of chick embryo at relatively precocious stages of synaptic and electrophysiological maturation[4]. A partial evaluation of the synaptosomal content in fractions has also been carried out.

Materials and methods. Subfractionation technique. Groups of chick embryos of 16 and 18 days of incubation and 2-day-old chicks were killed by decapitation and the 2 optic lobes rapidly dissected out and carefully deprived of mesencephali nuclei. Optic tecta were placed in cold 0.32 M sucrose plus 5 mM Tris-HCl (pH = 7.4) and homogenized at 10% w/v in a Teflon glass homogenizer. Differential centrifugation was carried out, based on the method described by Livett et al.[5] with slight modifica-

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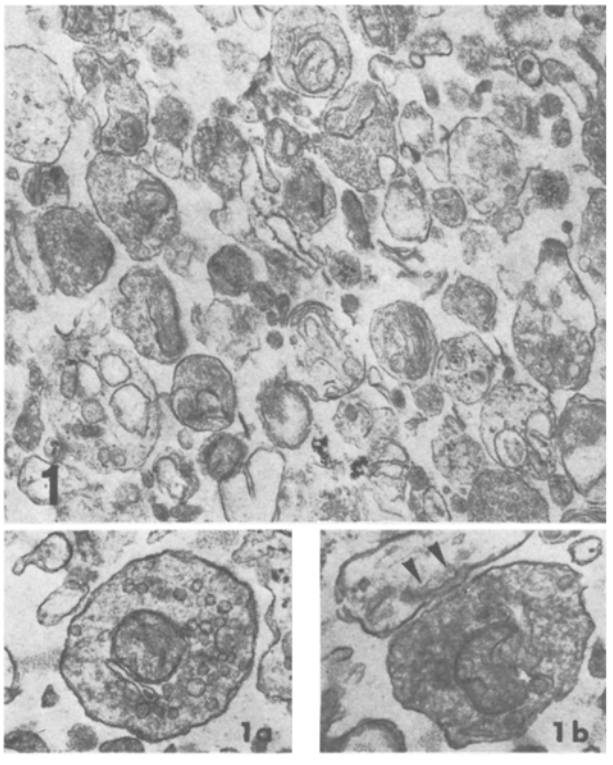

Fig. 1. Fraction B (between 7.5% and 13% Ficoll layers) isolated from chick embryos of 16 days of incubation. × 24,000. *a* and *b* Higher magnification showing the typical structure of synaptosomes from band B of 16-day-old embryos. Arrows point at postsynaptic membrane without definite thickenings. × 36,000.

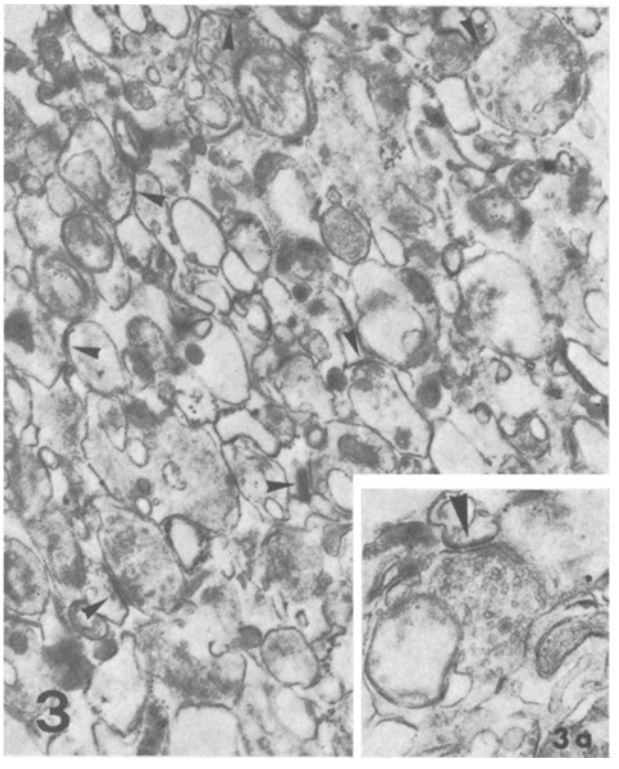

Fig. 3. Fraction B from chicks, showing the features of a typical synaptosomal fraction: nerve endings with many vesicles. Arrows point at postsynaptic membranes. × 25,000. *a* A well-defined postsynaptic thickening attached to a synaptosome from fraction B of chicks. × 40,000.

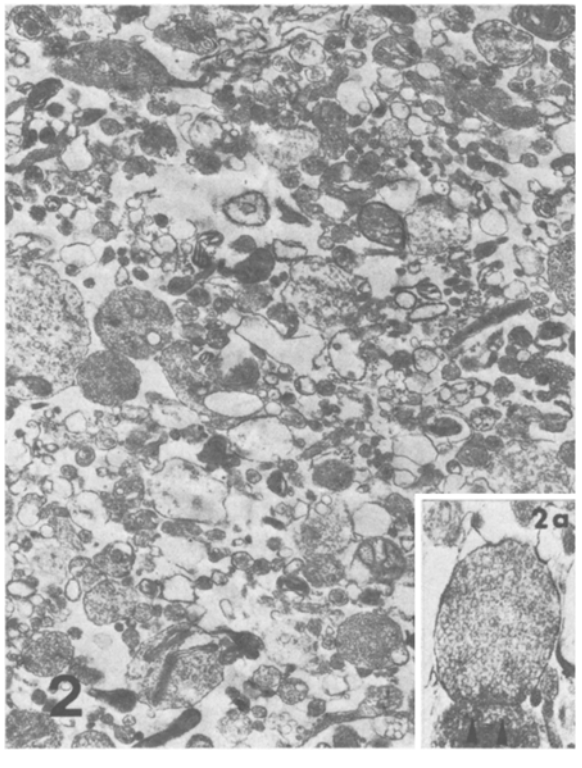

Fig. 2. Fraction B isolated from chick embryos of 18 days of incubation. × 16,000. *a* Synaptosome of fraction B showing an increased number of synaptic vesicles, and pre- and postsynaptic thickenings (arrows).

tions. A crude nuclear fraction (P_1) was removed by centrifugation at 1500 × g for 10 min. The pellet was washed twice and the pooled supernatants were centrifuged at 17,000 × g for 20 min in order to obtain crude mitochondrial fraction (P_2); 11,000 × g for 20 min were used in chick group experiments in order to avoid contamination by myelin fragments and disrupted axons. P_2 was resuspended in 6–8 ml of 0.32 M sucrose by hand homogenization and applied to a 2 steps discontinuous Ficoll-sucrose gradient, consisting of 10 ml layers of 13% (w/v) Ficoll (Pharmacia) in 0.32 M sucrose and 7.5% (w/v) Ficoll in 0.32 M sucrose. After centrifugation at 53,000 × g for 90 min (60 min for chicks), a synaptosomal fraction was obtained at the interfacie of the 7.5% and 13% Ficoll-sucrose gradient (band B). A second band (band A) was observed on the top of the gradient.

Electron microscopy. Small portions of fractions were fixed for 30 min in ice-cold 2.5% glutaraldehyde in 0.1 M phosphate buffer, spun on swing-out rotor, in order to obtain pellicles less than 500 µm thick, and postfixed, in situ, with 1% buffered OsO_4, for 1 h at + 4 °C. Pellicles were then dehydrated and embedded flat in a Epon-Araldite mixture. Sections were cut at right angle to the surface of pellicle through the whole pellet. They were stained with uranyl acetate and Reynold's lead citrate, and observed on a Siemens Elmiskop I A electron microscope, operated at 80 kV.

Quantitative evaluation of fractions. For each fraction we collected several series of pictures that covered the entire thickness of pellicle. A membrane-bound structure was defined as a synaptosome, according to Grove et al.[6],

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	16th		18th		2d	
	a	*b*	*a*	*b*	a	*b*
Homogenate	48.35	100	91.45	100	66.00	100
Crude mitochondrial fraction	9.60	19.86	15.84	17.32	13.68	20.72
A	2.88	30.00*	4.20	26.50*	3.58	26.17*
B	2.10	21.88*	6.31	39.80*	3.44	25.14*
C	2.26	23.54*	4.48	28.30*	6.52	47.66*
	c		*c*		*c*	
	55.6		43.6		66.8	

a Total amount of proteins per fraction (mg). *b* Percentage of proteins based on homogenate being 100%. * Percentage of proteins of each fraction based on crude mitochondrial fraction being 100%. *c* Percentage of synaptosome content in fraction B. For explanation see the text.

only if it contained 3 or more well-defined vesicles 300–500 Å in diameter. In order to eliminate the variable spaces between the structures from our calculation, we applied the method of Maunsbach[7], to compute the fractional area (A_a) occupied by synaptic structures. According to Weibel[8], fractional area is equivalent to the volume fraction occupied, in this case, by synaptosomes ($A_a = V_v$). The volume fraction has been expressed as a percentage, and used as a measure of the concentration of the synaptosomes in fraction B. Proteins were measured according to Lowry et al.[9].

Results. Synaptosomal fraction was collected at the interfacie of the 7.5% and 13% Ficoll-sucrose gradient at all the stages considered. At 16th day of incubation, this fraction was moderatly enriched in synaptosomes (figure 1) about 750 mμ in diameter. Nerve endings usually contained agranular vesicles 400 Å in diameter, and few mitochondria, occasionally neurotubules and large vesicles about 1000 Å in diameter. The observation of pre- and postsynaptic thickening was occasional. At 18th day of incubation, synaptosomes appeared about 800 mμ in diameter, full of vesicles 400 Å in diameter and showed variable electron density. Pre- and postsynaptic thickenings were often present (figure 2). Nerve endings isolated in chicks showed an increase in diameter (about 1 μm) and a low electron density. The mitochondria and vesicles content was sharply higher than in embryos. Largely asymmetrical synaptic thickenings were almost always present. The considered fraction appeared contaminated at all the stages. Contaminating structures were mainly free membranes, few polysomes and round bodies surrounded by a membrane, but devoid of vesicles. The contamination appeared lower in chicks than in embryos (see table).

No synaptosomes were detected in the pellet of the gradient in embryos, whereas a few disrupted nerve endings were observed in chicks. The fraction on the top of the gradient (A) was also observed at electron microscopy. In chicks it appeared enriched in membranes, some of which was recognizable as myelin membranes. In embryos some structures like synaptosomes, but deprived of pre- and postsynaptic thickenings, were observed with light membranes. Recovery of proteins in fraction B was about 22% of the total content of crude mitochondrial fraction in earlier embryos and 40% in older embryos. In chicks the percentage of total amount of the fraction was about 25%, more similar to 16-day-old animals than 18-day-old. The major content of proteins was measured in fraction A of the gradient in 16-day-old embryos, in the pellet in chicks, and in fraction B in 18-day-old animals.

Discussion. Augmentation in number of vesicles, increase in diameter and presence of symmetrical and asymmetrical synaptic thickenings are generally accepted as expression of synaptosomal maturation[10,11]. A progressive maturation of synaptosomes collected from fraction B, could be observed during development: before hatching synaptosomes showed a less content in vesicles, their diameter was inferior as the amount of recognizable synaptic thickenings. Synaptosomes with attached postsynaptic membrane were observed only in older embryos. These data are in accord with the observations of Abdel-Latif et al.[3], who showed similar structural changes in synaptosomes isolated from the whole brain of 14–16-day-old rat foetuses and postnatal rats. Also Hervornen et al.[12] observed that synaptosomes isolated from brain stem of 1-day-old and adult rats differed in vesicle content, diameter and presence of attached postsynaptic membrane. Lastly, morphology of synaptosomes of chicks is very similar to that reported by Östreicher and Van Leeuwen[13] on synaptosomes isolated from brain of 4–5-day-old chicks. A partial evaluation of enrichment in synaptosomial content in fraction B during development is done by data of synaptosome content in the table (see also methods). About 67% of volume of fraction B was due to synaptosomes in chicks, whereas only about 40–50% was measured in embryos. Quantitative data on the progressive enrichment of fraction B during development and immediately after hatching are in accord with the observations of Cantino and Sisto-Daneo[14], who reported that synaptogenesis in chick embryonic optic tectum occurs chiefly between the 18th day of incubation and hatching. However, a quantitative evaluation of synaptosomal recovery in fraction B is quite hard for many reasons. First, bodies surrounded by membrane, but devoid of vesicles were not calculated as synaptosomes. However, most of them could be nerve endings with few vesicles which had been sectioned without displaying the minimum number of synaptic vesicles. Consequently, an understimation of synaptic content of the fraction is probable in earlier stages. Secondly, the protein content is not conclusive, since the relative amount of lipids and proteins in synaptosomes presumably changes during maturation. In conclusion, these findings support the view that during development of chick embryonic tectum most of the synaptosomes isolated with Ficoll-sucrose gradient are immature nerve endings. They could be fruitfully used for studying biochemical and enzymatic properties of developing neural circuits, even if further studies are necessary to avoid contamination of synaptosomal fraction.

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