Pre- and Post-natal Development of the Spinal Cord: Increased Acetylcholinesterase Activity.* (31007)

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The onset of synaptic activity *in vitro* evidenced by bioelectric signs has been correlated with the first appearance of synaptic vesicles in electron micrographs of spinal cord cultures. Functional and morphological signs of synaptic activity occurred 3 days after explantation of 14-day fetal rat spinal cord(1). Studies *in vivo* have demonstrated that spinal cord convulsions can be elicited by direct stimulation of spinal cord in rat fetuses at 18 days of gestation (unpublished observations). In this study, spinal cord acetylcholinesterase (AChE)‡ activity during fetal and postnatal development of the rat was investigated in order to elucidate further the development of synaptic activity.

Materials and methods. Thirty pregnant Long-Evans rats were used. The presence of spermatozoa in the vagina was taken as criterion for fertilization, and the day in which they were found was considered day zero of gestation. The pregnant rats were given water *ad libitum* and were fed a white, synthetic diet developed in this laboratory to assure optimal nutritional conditions during gestation. After birth, offspring were kept with their mothers until weaning, at 21 days of age.

Although the uterus was examined daily for embryonal development, macroscopic identification of the developing offspring could not be adequately achieved until day 8 of gestation. From day 8 of gestation until 44 days after birth, an average of 6 animals per day was taken at representative days (Table I). From day 8 to day 10, the entire embryo was used for determination of the enzyme activity. From day 11 to day 13, the spinal column separated from the surrounding tissues was used. At day 14 and thereafter, the spinal cord was removed from the decapitated animal by means of the "toothpaste tube" method of Pomerat(2). All samples taken after birth were separated into cervical, thoracic and lumbar segments. Both gray and white matter of the spinal cord were used for enzyme determinations.

AChE activity was determined on the same

TABLE I. Changes in Acetylcholinesterase Activity and Protein Content of Spinal Cord of Rats During Pre- and Postnatal Development.

Age: days before birth	AChE activity, μM/min /g wet tissue	Protein content, mg/g wet tissue	AChE activity, μM/min /g protein
8*	.46 ± .10†	4.4 ±1.1	104 ± 22
9*	.51 ± .10	8.0 ± .6	63 ± 15
10*	.48 ± .01	10.4 ± .3	46 ± 6
11	.49 ± .05	10.3 ± .6	48 ± 5
12	1.22 ± .19	11.7 ± .6	104 ± 4
13	1.62 ± .19	13.7 ± .4	118 ± 14
14	2.22 ± .29	15.7 ± .3	141 ± 18
15	2.96 ± .38	16.1 ± .1	184 ± 24
16	4.11 ± .33	16.7 ± .6	246 ± 20
17	6.03 ± .25	18.7 ± .6	322 ± 13
18	8.66 ± .17	22.7 ±1.1	381 ± 8
19	9.70 ± .48	24.9 ±1.4	389 ± 19
Age: days after birth			
2 C‡	15.13 ± .30	33.2 ± 2.0	456 ± 9
T	13.28 ± .75	28.8 ± 2.6	461 ± 26
L	14.23 ± .70	31.5 ± 2.1	452 ± 22
9 C	12.66 ±1.13	73.9 ± 1.2	171 ± 15
T	13.17 ± .25	73.9 ± 1.8	178 ± 3
L	9.82 ±1.26	73.2 ± 1.8	134 ± 17
23 C	15.18 ±2.21	110.0 ±23.5	137 ± 20
T	13.89 ± .58	114.0 ± 9.9	122 ± 5
L	15.68 ± .43	115.0 ± 2.1	136 ± 4
44 C	9.65 ± .47	105.0 ± 8.9	92 ± 5
T	8.83 ± .51	100.0 ± 7.0	88 ± 5
L	10.86 ± .69	105.0 ± 6.9	104 ± 7

* Activity of total embryo.
† Mean ± standard error (S.E.), each mean represents 6 animals.
‡ Cervical (C), thoracic (T) and lumbar (L) segments of spinal cord.

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‡ The Commission on Enzymes of the International Union of Biochemistry has recommended that acetylcholine-acetylhydrolase (System No. 3.1.1.7) be the formal name, and acetylcholine esterase be the trivial name for the enzyme having the higher affinity for acetylcholine than for any other ester.

day of sacrifice by a modification of a colorimetric technique(3,6). Since protein content increases with age, the enzymatic activity of the spinal cord was expressed both as micromoles of acetylthiocholine hydrolyzed per minute per gram wet weight and per gram protein weight. Protein content was measured in the same sample used for enzyme activity by a colorimetric method(4).

Results. AChE activity expressed per weight of wet tissue increased progressively during fetal and postnatal development. During fetal development, from the relatively low levels up to day 11 of gestation, the activity increased by 60% at day 12, 59% from day 12 to day 15 and 51% from day 15 to day 17. Enzymatic activity in all segments of the spinal cord reached its peak at 2 days after birth, remained at this high level at 9 and 23 days of age, and was decreased at 44 days.

Although protein content increased progressively with age, when AChE was expressed per unit of protein, activity appeared to be decreased in the early prenatal (8 to 11 days) and postnatal (9 and 23 days) periods of development because of differences in rates of increase between protein content and enzymatic activity at these periods (Table I).

Discussion. The present data demonstrate a relatively high AChE activity in the spinal cord during late fetal development, while AChE activity in the whole brain has been shown to be low during this period(5). Peak AChE activity of the spinal cord was attained at 2 days after birth, whereas in other CNS areas the peak activity was reached about 24 days after birth(5,6). Since AChE has been used as evidence for the presence of ACh (7), cholinergic systems must develop earlier in the spinal cord than in higher CNS centers.

It is not evident from these data whether the measured AChE activity is contributed primarily by neurons, glial cells or nerve fibers. Although it is known that AChE activity is highest in neurons(7), the contribution of glial cells and nerve fibers to the measured AChE activity cannot be excluded. However, bioelectric activity(1), appearance of synaptic vesicles(1), and presence of AChE activity at an early period of gestation suggest prenatal synaptic function.

Summary. Acetylcholinesterase (AChE) activity of the spinal cord in rats was determined colorimetrically during pre- and postnatal development. The enzyme activity increased progressively from 11 days before birth to 2 days after birth. The presence of AChE activity at the early prenatal period is correlated with morphological and functional development of the synapse in the rat spinal cord.

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