Alterations in central latency, motoneurone number and blood volume of spinal cord of the aging rat

G. SANT’AMBROGIO, D. FRAZIER, R. REED, AND L. L. BOYARKY
Department of Physiology, University of Kentucky, Lexington, Kentucky


Measurements were made of the central delay of the flexor reflex, the number of motoneurones in the ventral horn of the lumbar region of the cord, and the tissue blood volume of the lumbar region of the cord. There is a 50% decrease in tissue blood volume between the ages 450 and 550 days. A loss in the number of motoneurones of the cord of about 30% also occurs between the same ages. Central latency increases about 60% from a value of 3.51 msec to 5.82 msec between the ages 550 and 750 days. This rise in central latency takes place after the blood volume decrease and the loss in number of cells. It is suggested that the primary event in the aging of the nervous system of the white rat is a restriction of the vascular bed; with changes in latency secondary to the loss in neurones. These results are also taken as evidence that local, intrasegmental changes in the cord as well as alterations of suprasegmental downflow play an important role in aging of the spinal cord.

EXPERIMENTAL PROCEDURE

Changes in the nervous system during aging have been the object of a number of recent studies. In one type of investigation, basic aspects of senescence such as nerve conduction velocity (1, 2), monosynaptic delay (3) and motoneurone cell number loss (4) have been studied. Another group of investigations has involved more complex phenomena such as the startle reaction time of the rat (5) and central delay in the reflex response of man (6). Both the simple and complex phenomena have shown pronounced changes with age.

A prominent theory of aging asserts that it is the result of mechanical or metabolic wear and tear to particular tissues and organs. The breakdown of a given system is the final result of the random failure of component elements of that system. There is considerable evidence that the circulatory system of man and the lower animals shows degenerative changes with age (7-9) and it is reasonable to assume that this system is the first major entity to fail in the organism. The present work is an attempt to relate changes in two properties of the spinal cord of the white rat to alterations in the circulation of that organ. Changes in the latency of the polysynaptic flexor reflex and motoneurone cell number have been correlated with the tissue blood volume of the spinal cord during aging. If a restricted blood supply to the spinal cord results in nervous system damage, tissue blood volume, a measure of circulatory supply, should decrease before reflex latency or motoneurone number alter. This prediction is supported by the data of our experiments.

EXPERIMENTAL PROCEDURE

The male white rat of the Wistar strain was the subject in all experiments. Rats were purchased at around 100 days of age and raised in the laboratory to an appropriate age. These animals also served as breeding stock to obtain very young animals (50 days).

Reflex latency was measured by a direct method. The rats were anesthetized with ether and the spinal cord completely transected at the level of the first thoracic vertebra. Reflexes were tested 15 hours following transection. The suralis and semitendinosus nerves were exposed. The suralis was stimulated and the action potential of the reflex response recorded from the semitendinosus nerve so that the total latency was procured. Conduction time was subtracted from total reflex latency to obtain central delay. Potentials were amplified with a Grass P-4 preamplifier and Dumont 364H oscilloscope. A stimulus of 0.3 sigma duration, supermaximal for A fibers was used. Photographs of each potential and reflex were obtained in triplicate with a Polaroid Land camera. Reflex measurements were obtained from 49 rats consisting of six age groups whose mean ages, in days, within 10% were: 50(9), 100(14),

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200(8), 400(6), 550(5) and 750(7). The number in each group is given in parentheses.

The lumbar region of the spinal cord from L₁-L₃ was removed from the animals on which reflex measurements were made. Cords were fixed in 10% formalin, imbedded in paraffin, stained with thionine, and sectioned at 50 μ. Counts were made of all the large motoneurones of both ventral horns on five or more sections per animal and the results tabulated as mean counts per section per animal. The age groups and number of animals in each group (in parentheses) were: 50(5), 100(7), 400(6) and 550(5) days.

Spinal cord blood volume was determined with a separate series of animals. Two rats used as donors were injected with 100 μc of Fe⁵⁹ intraperitoneally. Two weeks later 1 cc of blood was withdrawn from a donor, heparinized and injected i.v. into a group of rats of a particular age group. Ten minutes after the injection, the spinal cords were exposed in the lumbar region after high cord transection and dry-ice alcohol mixture (−72°C) poured over the cord. Control experiments showed that the temperature of the cord fell to freezing within 3 seconds. The frozen region was removed, weighed, dissolved in concentrated HNO₃ and counted with an end-window geiger tube and scaler. Samples of blood were obtained just before freezing the cord and the radioactivity counted. Tissue blood volume was calculated from the ratio counts/cubic centimeter blood to counts/milligram tissue and is expressed as cubic centimeter blood/milligram tissue. Four groups of animals were used in the blood volume study whose mean ages with the number of animals per group (in parentheses) were: 100(11), 200(12), 400(11) and 550(7) days.

The one-sided t test was used to evaluate statistical significance between different age groups.

RESULTS

Growth pattern of the population. Measurements of body weight and sciatic nerve lengths were made of each animal in order to obtain an estimate of the onset of maturity in the population. In figure 1, the weight of the animals and the total length of the sciatic nerve are plotted for each age group. The data indicate that maturity, as measured by both these criteria, is attained at about 400 days of age; the greatest rate of change obtained between the 50- and 100-day-old animals. The pattern of growth in the present group of Wistar animals appears to be like that reported for a larger group of animals (10).
Conduction velocities. The conduction velocities as a function of age for the afferent and efferent pathways are shown in figures 2 and 3. Efferent conduction velocity ranged from 41.4 to 52.0 m/sec. Afferent conduction velocities varied from 33.9 to 50.6 m/sec. There is a statistically significant (P < .05) rise of afferent conduction velocity between the 50- and 100-day-old animals.

Central delay. Figure 4 is a graph of the central reflex time as a function of age. The vertical bars denote the standard error of the mean for each mean delay. The coefficient of variation (standard error/mean) was 10% for all groups but the oldest, for which the coefficient was about 40%. Central delay decreases during the period 50-100 days from 4.85 to 3.52 sigma; a change which is significant at the 0.02 level. Between the ages 100 and 550 days there is no significant alteration in reflex delay. Between the ages 550 and 750 days there is an increase in central delay of 60% from 3.51 to 5.82 sigma; a change statistically significant at the 0.02 level. The significant changes in central latency occur, therefore, in the very young and very old age groups.

The injection of caffeine (50 mg/kg i.p.) causes a two- to threefold increase in the amplitude of the reflex response. This is taken as evidence that the central delay in our preparation is being estimated for only a fraction of the total motoneurone pool of the flexor reflex.

Motoneurone cell number. Figure 5 shows the change in motoneurone cell number for four groups between the ages 50 and 550 days. Cell number (in arbitrary units and per section) decreases from 39.1 at 50 days to 26.9 at 550 days. The decrease in cell number is only statistically significant between the 400 and 550 age groups (P < 0.1). The earlier, apparently linear decrease in cell number, may be attributed to growth in length of the spinal cord. Measurements of spinal cord lengths at various ages show that the cord length, as measured from atlas to cauda equina tip, increases 10% between 100 and 200 days, remaining constant thereafter.

Spinal cord blood volume. Figure 6 summarizes the measurements of spinal cord blood volume of four age groups containing different animals than those used for the delay and cell number studies. Spinal cord blood volume remains the same in the three age groups, 100, 200 and 400 days of age but drops sharply from a value of 0.14 cc/mg of spinal cord to 0.08 cc/mg at 550 days of age. This drop of 50% in tissue blood volume is significant at the 0.01 level. Variability was low as indicated by a coefficient of variation of 5% for the 550-day age group. No analyses were successful on the 50-day-old group.

DISCUSSION

The changes in latency described above for the polysynaptic flexor reflex are quite unlike those reported to take place with the monosynaptic reflex (3). In the monosynaptic system there was a continuing, linear increase in central latency beginning with the youngest animals and continuing into old age. The latency of the flexor reflex, however, decreases during maturation between 50 and 100 days, remains constant to about 550 days and rises between 550 and 750 days. Similar results for a polysynaptic reflex have been reported to occur in humans in a study of cutaneous reflex responses (6). Latency of the cutaneous reflexes is greater for the very young and very old subjects than for the middle-aged. In more complicated tasks, such as auditory and visual reaction times, older subjects show a longer delay than younger subjects (11). The response time of rats to electric shock and sudden noises shows an increase only in groups older than 400 days (5). As would be anticipated, the polysynaptic flexor reflex offers a better model for interpreting aging in the central nervous system than the physiologically simpler monosynaptic reflex.

The decrease in motoneurone cell number in the rat spinal cord is in agreement with the substantial body of evidence demonstrating a loss of cells within the nervous system during aging. Such decreases have been shown for the Purkinje cells of the rat and human cerebellum, hu-
man cerebral cortical neurones, human spinal ganglion cells and motoneurones of the mouse spinal cord (4). Nerve fibers appear to be more resistant to senescent deterioration. Most workers report little change in number of fibers of peripheral nerve (2); nor have we detected any major changes in nerve conduction velocity. There is a rise in velocity in young, maturing animals. Similar results have been reported for sciatic nerves in vitro (2).

Circulatory changes in the rat with age have not been extensively studied. It is known that the rat does not suffer from arteriosclerosis but is more susceptible to periarteritis, with the onset of the disease occurring at about 400 days of age (8). Blood pressure is not different in very young and old rats. Leucocytic counts show a decrease after 600 days of age (12). There have been no studies of blood volume changes with age in the rat but measurements of spinal cord blood volume for apparently middle-aged rats are half of the value we have found (13). The difference in results is obviously explained by the diverse methods, the quoted workers having dipped the entire animal into liquid air so that freezing took 30 seconds compared with about 3 seconds in our experiments.

More to the point are the studies of human cerebral circulation with age. In the human, cerebral blood flow decreases and cerebrovascular resistance increases with age (7). Unlike the spinal cord blood volume of the rat, the cerebrovascular measures in the human change continuously without any sharp onset. The same author has reported a continuous decrease of cerebral oxygen consumption with age. We have no information on the variation of rat cerebral or spinal oxygen consumption with age with which to compare the human data. It is possible that senescent events in the rat spinal cord are basically different from those of the human cerebrum. The diminution in flow whether continuous or abrupt, however, remains unexplained. Since there is no arteriosclerosis in the rat, the decreased size of the vascular bed of the spinal cord may be the result of tonic vascular alterations rather than the result of the deposition of material in the vascular wall as appears likely in the human being.

The three properties of the spinal cord which were studied are summarized in figure 7. It is clear that the first major change to occur is a fall in spinal cord blood volume between 400 and 550 days. The change amounts to about a 50% decline in tissue blood volume. Subsequently, in animals older than 550 days of age, there is a 60% increase in the central latency of the flexor reflex. The decrease in spinal cord cell number occurs concomitantly with the decrease in tissue blood volume. Both the latency and cell number change conform to the hypothesis that the primary change in the nervous system of the rat during aging involves a disturbance of the circulation within the spinal cord. The diminution in blood volume, which occurs before the other changes, suggests that the first event among the three measured here is a restriction of circulation. This restriction in vascular supply results in either an anoxia or malnutrition of the cord or a combination of both conditions. Such a noxious state then leads to the death and disappearance of cells. Presumably, the loss of cells and the consequent decrease in segmental facilitating activity accounts for the prolongation of central delay.

It has been argued that the increase in latency of cutaneous reflexes in human beings is a result of diminished facilitating influences from suprasegmental regions and that intrasegmental or synaptic events are not impaired with age (6). Our results, demonstrating that circulatory abatement occurs before the nervous system is affected, tends to diminish the reliability of this conjecture. Changes in the circulation of a spinal segment leading to anoxia or nutritive deficiency certainly imply that the chemistry of the synapse could be affected. Similar circulatory changes may occur in the human spinal cord since a reduced cerebral circulation has been demonstrated in the older human being. Furthermore, the evidence of the present study and of previous work (4) shows that there is a definite diminution in the cellular population of the spinal cord of older populations of several species. Loss of the low-latency elements of the polysynaptic chain appears, then, to be a likely explanation of the prolonged latency of the flexor reflex in older animals. It is not possible, at present, to assess the relative roles of supra- and intrasegmental factors in the prolongation of latency during aging in the nervous system.

REFERENCES