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Age-Related Changes in the Electrical Activities of Rat Sympathetic Ganglion Cells *In Vitro*

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The supeior cervical ganglia with the preganglionic nerve fibers were isolated from albino rats at the various ages; from 1 day to 2 years after birth. The membrane potential of individual ganglion cells was recorded with intracellular micro-electrode techniques. Both amplitudes of resting and action potentials were smaller in young rats. Membrane resistance of the cells tended to decrease with increase in age, reflecting growth of cell size. Amplitude and frequency of the miniature excitatory postsynaptic potentials (mEPSPs) in the new-born rats were 2.5 and 11 times as large as those in the matured rats, respectively. Conduction velocity of the preganglionic nerve fibers increased as a function of age. In almost all of the impaled cells in the matured rats, single supramaximal shocks to the preganglionic nerve fibers produced EPSPs with initiation of action potentials. Whereas, in some cells in new-born rats, similar stimuli produced EPSP alone but no action potential. Even in this type of cells, however, tetanic stimuli caused summation or facilitation of EPSPs, leading to initiation of action potential. In the present study, it was also found that response to exogeneously applied acetylcholine was greater in the new-born rats than in the matured ones.

Key words : age-related change, electrical activities, sympathetic ganglion cells, rat

1. Introduction

A number of studies have previously been reported for the age-related changes in electrical activities of skeletal muscles fibers of developing rats and mice as to resting potentials (RPs) and action potentils (APs)[1-2], conduction velocities of innervating motor nerve fibers[3], and transmission at end-plate[2,4]. In the skeletal muscle fibers of the new-born animals, the RPs were lower, the amplitudes of APs were smaller, and the conduction velocities of the innervating motor nerve fibers were slower as compared to those in the adults. The configuration of end plate potentials in the skeletal muscle fibers at birth was found to be complex, and become simpler during the second week after birth.

In the present study, using the sympathetic ganglion preparations isolated from the new-born rats, the electrical activities in the sympathetic ganglion cells in vitro and synaptic transmission from the preganglionic nerve fibers to the ganglion cells were examined by intracellular micro-electrode techniques. And the age-related changes in the activities during the growth of rats were also examined.

2. Methods

The superior cervical ganglia with the prganglionic nerve fibers were isolated from albino rats at the various ages from one day to 2 years after birth, under ether anesthesia. Preparations were mounted in a constant temperature bath (34-36 ℃) perfused with a modified Krebs solution, as described previously[5]. A single glass micro-elctrode filled with 0.6M K_2SO_4 was used to record intracellulary the membrane potential of individual ganglion cells. The preganglionic nerve fibers were stimulated with two rings of Pt wire electrodes to evoke orthodromic responses in ganglion cells. The membrane resistances of the cells were measured by intracellular application of current steps through a Wheastone bridge circuit[5].

3. Results

As shown in Fig.1, the general feature in electrical activities of the sympathetic ganglion cells in vitro was similar in many respects regardless of ages of rat. For example, a weaker stimulation of the preganglionic

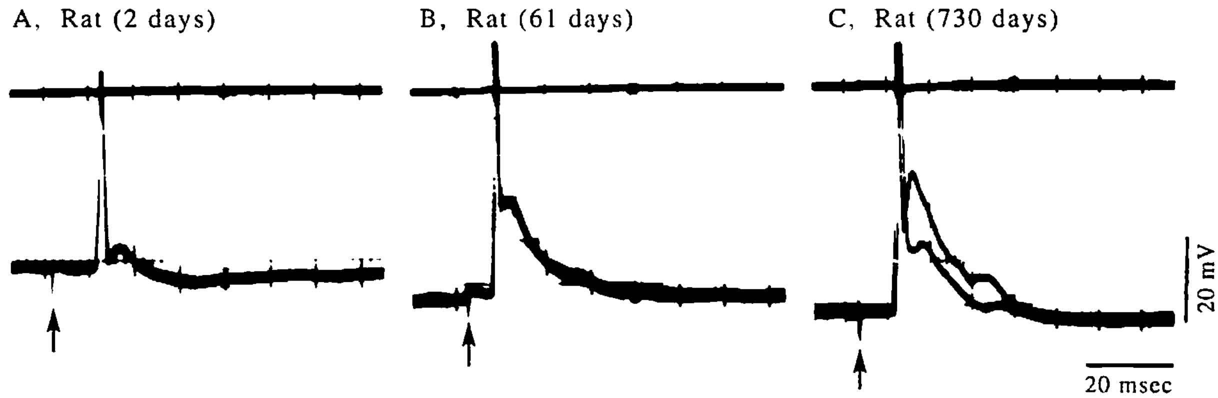

Fig.1. APs evoked by suparamaximal preganglionic nerve stimulation. Records A, B and C were obtained from the sympathetic ganglion cells of rats at 2, 61 and 730 days after birth, respectively. Arrows indicate stimulus artifacts.

nerves evoked EPSP (s) and increasing the stimulus strength gave rise to EPSP (s) large enough to initiate an action potential in the majority of cells tested, even in the cells of new-born rats.

One of the age-related changes in the electrical activities was noticed, however, in the temporal dispersion of EPSPs which, in response to single stimulation, increased with increase in age(Fig.1C). This indicates development of convergence of preganglionic nerve fibers with different conduction velocities.

The other age-related changes in the electrical activities were found in quantitative mesurements as to the values for the amplitudes of RPs, APs and membrane resistance, amplitudes and frequency of miniature exitatory postsynaptic potentials (mEPSPs), and the values for the velocities of the most rapidly conducting impulses in the preganglionic nerves.

Table 1 summarizes the results of quantitative measurements of the electrical activities of rat sympathetic ganglion cells at 1-2, 3-6, 9-20, 40-62 and 730 days after birth. Although there was a large variability in the values obtained for individual ganglion cells, both of the RPs and amplitudes of the APs were, on the average, smaller in the younger rats. The values for either RPs or APs were likely to reach a level of those in adult rats at ages between 2 weeks and 2 months after birth. On the other hand, both RPs and APs in the 730 days old rats did not significantly differ from those of 2 month old rats. Data for the input resistances of cell membranes were not obtained sufficiently, but there was a tendency in the values to decrease with an increase in age. This may reflect to some extent an increase in diameters of the developing ganglion cells.

As shown in Fig.2 and Fig.3, amplitude and frequency of the mEPSPs in the new-born rats were larger than those in the old rats. On the average, the amplitude and frequency of mEPSPs in the new-born rats were 2.5 and 11 times as large as those in the old rats, respectively.

Table 1. Electrical activities in vitro of the sympathetic ganglion cells of rats examined at the various days after birth.

Age (days)	Resting Potential (mV)	Action Potential * (mV)	Membrane Resitstance (M Ω)	mEPSP Amplitude (mV)	mEPSP Frequency (Hz)	Conduction Velocity * (m/sec)
1 –2	43 ± 5 (9)	50 ± 9 (6)	75 (2)	2.8 ± 1.0 (7)	1.32 ± 1.33 (6)	0.5 ± 0.1 (11)
3 –6	44 ± 4 (11)	50 ± 9 (8)	84 ± 44 (5)	–	–	0.5 ± 0.2 (7)
9 –20	46 ± 5 (9)	59 ± 8 (9)	70 ± 35 (4)	–	–	0.6 ± 0.1 (5)
40 –62	52 ± 7 (29)	63 ± 9 (29)	69 ± 30 (14)	1.4 (3)	0.16 ± 0.16 (5)	0.8 ± 0.4 (17)
730	49 ± 4 (13)	61 ± 7 (13)	54 ± 26 (11)	1.1 ± 0.4 (7)	0.12 ± 0.08 (8)	1.1 ± 0.3 (15)

Values are means ±S.D.. Numbers in parentheses indicate number of cells analysed.

*, Amplitudes of the action potentials evoked by the preganglionic nerve stimulation.

*, Velocities of the most rapidly conducting impulses in the preganglionic nerve, approximated by an estimation from latency of the first EPSP and distances between stimulationg and recording electrodes.

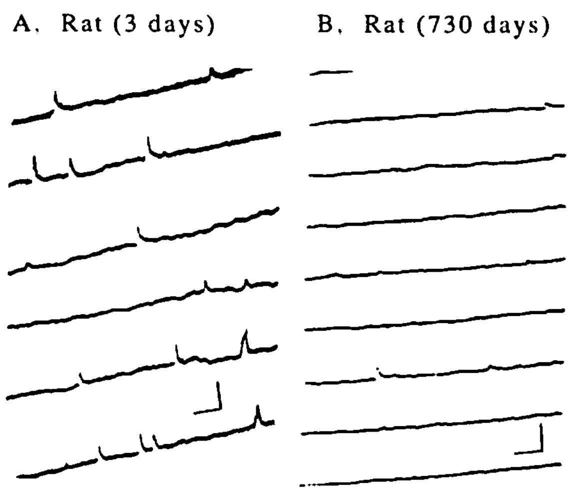

Fig.2. Examples of mEPSPs recorded from a ganglion cell of rats at 3 days (A) and 730 days (B) of age. Both records continue from top to bottom. Note that larger mEPSPs occurred at higher frequency in A(3 day old rat), as compared to those in B (730 day old rat).

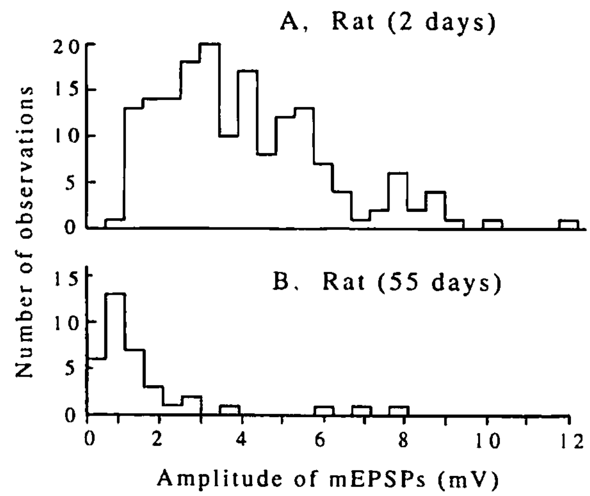

Fig.3. Examples of histograms for amplitudes of mEPSPs analysed in a ganglion cell of rats at 2 days (A) and 55 days of age (B). The mean values for amplitude and frequency were 4.2 ± 2.0 mV and 0.84 Hz in A, and 1.8 ± 1.8 mV and 0.44 Hz in B, respectively.

Some cells in the new-born rats showed spontaneous discharges of APs from the mEPSPs occuring at a high frequency.

Velocities of the most rapidly conducting impulses in the preganglionic nerve fibers were found to increase as a function of age. The average value for the velocities at 730 day after birth was approximately twice as large as that in the new-born rats. It was also noticed that some proportions of the cells examined in the old rats were innervated by the nerve fibers of relatively slower conduction velocities. This yields larage S.D.s of the mean values in the old rats.

When the preganglionic nerve fibers were stimulated by a pulse of supramaximal strength, almost all of the cells tested responded with initiation of APs. However, in some cells in the new-born rats, the stimulation could not produce any AP but EPSPs alone, as shown in Fig.4A. Each EPSP in response to the successive stimulation by a strong and constant pulse fluctuated in size. Nevertheless, when the nerve fibers were stimulated by a

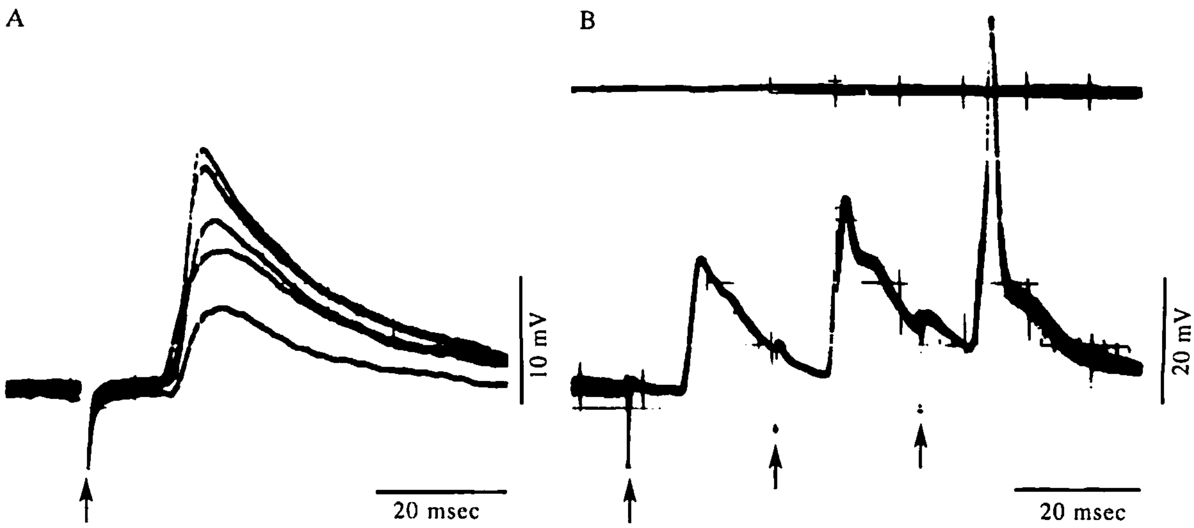

Fig.4. EPSPs elicited by supramaximal preganglionic nerve stimulation in a ganglionic cell of a rat at 1 day after birth.
A, a single supramaximal stimulation of the preganglinic nerves could not produce any action potential but produced EPSPs only, which fluctuated in size in response to the successive (5 in this case) stimulation. B, in the same cell, summation of EPSPs in response to tetanic stimulation resulted in initiation of an action potential. Arrows indicate stimulus artifacts.

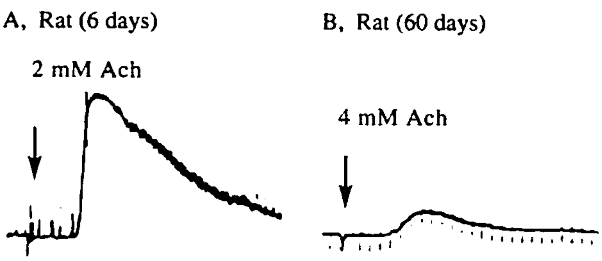

Fig.5. Comparison of amplitudes of Ach-potentials in the ganglion cells between rats at 6 days (A) and 60 days (B) of age. Arrows indicate the time of Ach application.

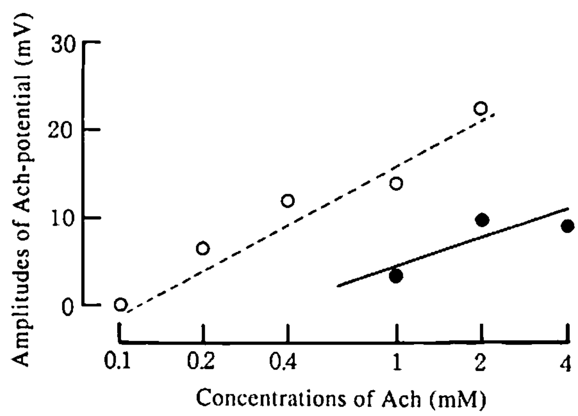

Fig.6. Dose-depended amplitudes of Ach-potentials examined in the ganglion cells of rats at 1-6 days (○) and 51-73 days (●) of age.

train of the pulses, in all those cells tested there occurred summation or facilitation of EPSPs which led to initiation of APs, as shown in Fig.4B.

In the present study, responsiveness of the ganglion cells to exogenously applied acetylcholine (Ach) were also examined. As shown in Fig.5, in response to injection of Ach into the perfusion current, a transient depolarization (Ach-potential) occurred in the ganglion cells. The time-courses of Ach-potential were more or less similar regardless of ages of rats. Sensitivity of the ganglion cells to exogenously applied Ach, however, was approximately 10 times greater in the new-born rats than in the matured ones (Fig.6).

4. Discussion

Harris and Luff[1)] have reported for the mice skeletal muscle fibers that the RPs increase with the age of the animals, reaching a level higher by about 20 mV at 16 weeks after birth than those in the new-born. The present obsevation of an increase in the RPs, though to a smaller extent, of the developing sympathetic ganglion cells is in a general agreement with the previous finding. As expected, the increase in RPs was accompanied with increase in amplitudes of the APs.

The temporal dispersion of EPSPs in response to single preganglionic nerve stimulation increased with increase in age, indicating development of convergence of preganglionic nerve fibers with different conduction velocities. This supports the view that the synaptic connections become more complex ones with age, and/or there is differentiation of the conduction velocities in the preganlionic nerve fibers innervating the cells. In contrast to the observation of the development in the neuro-neuronal connection, the neuro-muscular connection in rats was found to be complex at birth and become simpler during second week after birth[4] .

It was also noticed in the present study that some proportions of the ganglion cells examined in the old rats were innervated by the nerve fibers of relatively slower conduction velocities. This may reflect that in the old rats, some proportions of the preganglionic nerve fibers undergo functional deteriorations.

Amplitudes of the mEPSPs were larger in the new-born rats. This may be contributed to: (1) a larger input resistance of the cell membranes, as expected from a smaller size of the cells: (2) a lower activity of acetylcholinesterase in the synapses, as described previously in a neurochemical study of the chick ganglion cells by Sorimachi and Kataoka[6] . As to the frequency of spontaneous transmitter release, Diamond and Miledi[7] have reported for the diaphragm muscle fibers that the miniature end plate potentials in the foetal and new-born rats occurs at a lower frequency rate than in the adult. In contrast, the mEPSPs of the sympathetic ganglion cells in the new-born rats were found in the present study to occur at a higher frequency rate than in the adult. This discrepancy, however, could not be readily explained.

Lewis[3] has measured conduction velocities in the left phrenic nerve of developing rats from birth to 6 months of age. His results seem to show several times larger increase in the velocities with age than that observed presently in the preganglionic nerve fibers of the sympathetic nervous system. This may indicate a larger increase in diameters of the motor nerve fibers than in diameters of the sympathetic ones.

Since almost all of the cells tested, even in the new-born rats, responded with initiation of APs to the supramaximal preganglionic nerve stimulation, it could be said that a functional synaptic connection would have already been established even in the new-born rats. In those cells of the new-born rats in which the stimulation by a single pulse could not produce any AP, the stimulation by a train of the pulses did produce APs. Therefore, even in this type of cells there was a possibility for that activities of the central nervous system could be transmitted to some extent through the synapses to peripheral organs, when the efferent impulses from the central nervous system to peripheral organgs come down at high frequency.

In the present study, it was also found that Ach-potential was greater in the new-born rats than in the matured ones. The reasons for this observation seem similar to the ones for that the size of spontaneous mEPSPs were greater in the new-born rats, i.e., (1) a larger input resistance of the cell membranes and (2) a lower activity of acetylcholinesterase in the synapses in the new-born rats, as described above. Another possibility exists in the assumption that the responsiveness of the membranes or Ach-receptors are so greater, producing greater current flow due to exogeneously applied Ach. This may be said to be one of the most striking features of the ganglion cells in the new-born rats.

5. Conclusion

An electrophysiological study with intracellular micro-electrode techniques found that, in the sympathetic ganglion cells of new-born rats, amplitudes of RPs and APs are lower, membrane resistances are greater, and the conduction velocities in the preganglionic nerve fibers are slower than those in the matured rats. It was also found that mEPSPs are greater in size and more frequent at occurrence rates, and the amplitudes of Ach-potentials are greater in the new-born rats than in the matured ones. In some of the gangion cells of the new-born rats, the stimulation by a single pulse could not produce any AP, but the stimulation by a train of the pulses did produce APs, indicating a possibility for that activities of the central nervous system could be transmitted to some extent to peripheral organs through even this type of synapses. The present study indicates that electrical activities of the isolated sympathetic ganglia reach a level of adult rats at between 2 weeks and 2 months after birth. A functional synaptic contact of a simple form does exist already in the new-born rats, and continues to develope to more complex ones during their life from birth to more than 2 months.

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