ity of the neuron and the duration of the excitatory component of their response to the mediator is shown in Fig. 1, in which curve 1 shows the distribution of the test neurons by the durations of these components, whereas curves 2 and 3 show the type and abundance of the time course of spontaneous and evoked activity respectively. Values of the ordinate at each point on curves 2 and 3 are the algebraic sum of the number of neurons exhibiting an increase (above the zero for each graph) or a decrease (below the zero) of activity of a particular type. It will be clear from Fig. 1 that the probability that a neuron exhibits a time course of spontaneous or evoked activity during the repeated action of ACh is greatest when the duration (which is strictly specific for each nerve cell) of the excitatory component of their response is 3.2, 8.1, and 13.5 sec, and that the duration of excitation has a different relationshp to the direction of the time course of spontaneous and evoked activity.

The results of investigation of cholinoceptive neurons thus demonstrate differences in plasticity of inputs of the same chemical nature. Analysis of the plasticity of inputs of different chemical nature will provide a deeper insight into the mechanisms lying at the basis of learning and memory.

LITERATURE CITED

1. B. I. Kotlyar, A. A. Myasnikov, A. D. Kartaev, et al., Nauch. Dokl. Vyssh. Shkoly, Biol. Nauki, No. 9, 52 (1984).
2. B. I. Kotlyar and Yu. S. Ovcharenko, Zh. Vyssh. Nerv. Deyat., No. 5, 971 (1976).
3. R. I. Kruglikov, Neurochemical Mechanisms of Learning and Memory [in Russian], Moscow (1981).
4. G. N. Opeinik, Usp. Fiziol. Nauk, 9, No. 1, 106 (1978).
5. G. G. Khludova, A. A. Myasnikov, and B. I. Kotlyar, Nauch. Dokl. Vyssh. Shkoly, Biol. Nauki, No. 12, 40 (1984).
6. K. Krnjevic, Physiol. Rev., 54, 418 (1974).
7. K. Krnjevic and J. W. Phillis, J. Physiol. (London), 166, 296 (1963).
8. K. Krnjevic and J. W. Phillis, J. Physiol. (London), 166, 328 (1963).
9. J. W. Phillis and D. H. York, Brain Res., 5, 517 (1967).
10. J. W. Phillis and D. H. York, Brain Res., 10, 297 (1968).
11. T. W. Stone, J. Physiol. (London), 255, 485 (1972).

EFFECT OF CALCIUM ON FORCE-FREQUENCY RELATIONSHIP AND RESTING POTENTIATION IN THE MYOCARDIUM OF ADULT AND OLD RATS

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The adaptive powers of the cardiovascular system are considerably reduced during aging [3, 7, 8]. Elucidation of the primary mechanisms lying at the basis of these changes is essential for the development of effective ways of increasing the adaptive capacity of the aging organism. Dependence of the developed tension, and the rate of contraction and relaxation on frequency enables the heart muscle to maintain optimal contractile activity during changes of rhythm. The rat myocardium is characterized by a negative chronoionotropic relationship [4, 13, 15]. The study of age changes in this relationship has shown that the velocity-force parameters of heart muscle during an increase in the frequency of stimulation to 5.0 Hz change less in old rats than in adult rats, and that the potentiation of contractions by an interval of rest also is depressed in the old myocardium [4]. The important role of Ca^{++} ions in the realization of chronoinotropic relations is well known [1, 2, 6], and changes in

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TABLE 1. Biomechanical Parameters of Rhythmic and Test Contractions of Myocardium of Adult (A) and Old (B) Rats in Different External Ca^{++} Concentrations

$CaCl_2$ concentration, mM	Frequency Hz	Developed tension, g/mm²		Rate of rise of tension, g/mm²·sec		Rate of fall of tension, g/mm²·sec	
		A	B	A	B	A	B
0,30	0,1	0,84±0,09	0,38±0,04	10,79±1,15	4,63±0,66	6,94±1,09	3,52±0,75
		(1,07±0,12)	(0,42±0,04)	(12,87±1,49)	(5,05±0,62)	(8,42±1,58)	(3,57±0,66)
	1,0	0,21±0,02	0,26±0,03*	5,25±0,78	3,82±0,60*	3,44±0,62	2,28±0,63*
		(0,74±0,09)	(0,38±0,05)	(12,29±1,25)	(5,04±0,74)	(7,29±1,23)	(3,98±0,70)
	2,0	0,12±0,01	0,10±0,02*	2,71±0,26	2,88±0,26*	2,67±0,71	1,70±0,36*
		(0,64±0,07)	(0,33±0,04)	(12,21±0,79)	(7,63±0,43)	(9,70±1,02)	(3,57±0,71)
1,25	0,1	1,57±0,17	0,61±0,02	23,04±2,13	9,69±0,94	14,96±1,50	6,00±0,78
		(1,56±0,18)	(0,61±0,02)	(23,14±2,13)	(9,86±0,95)	(15,07±1,50)	(5,96±0,79)
	1,0	0,67±0,07	0,35±0,06	12,85±1,70	6,78±0,99	7,43±1,18	3,49±0,47
		(1,28±1,13)	(0,57±0,05)	(18,79±1,95)	(8,21±0,98)	(14,30±1,45)	(6,32±0,98)
	2,0	0,49±0,07	0,26±0,03	10,10±1,38	5,44±0,50	5,63±0,99	3,37±0,59
		(1,40±0,16)	(0,60±0,08)	(21,74±2,00)	(8,66±0,49)	(15,25±1,05)	(5,82±0,84)
5,00	0,1	1,41±0,18	0,61±0,06	24,22±3,08	8,72±0,33	16,73±2,55	6,37±1,03
		(1,39±0,19)	(0,65±0,06)	(23,58±3,35)	(9,74±0,38)	(16,02±2,38)	(8,11±1,55)
	1,00	0,98±0,11	0,55±0,07	21,72±1,92	10,12±0,63	14,62±2,30	7,78±1,07
		(0,93±0,10)	(0,60±0,06)	(22,84±2,88)	(10,04±0,60)	(16,67±1,88)	(7,66±1,10)
	2,00	1,00±0,11	0,48±0,04	19,95±1,99	7,48±0,47	13,84±1,99	5,06±0,96
		(1,12±0,12)	(0,57±0,01)	(22,30±1,93)	(8,02±0,48)	(14,11±2,31)	(4,50±0,88)

Legend. *) Differences between old and adult animals not significant. Values of parameters for test contractions given in parentheses.

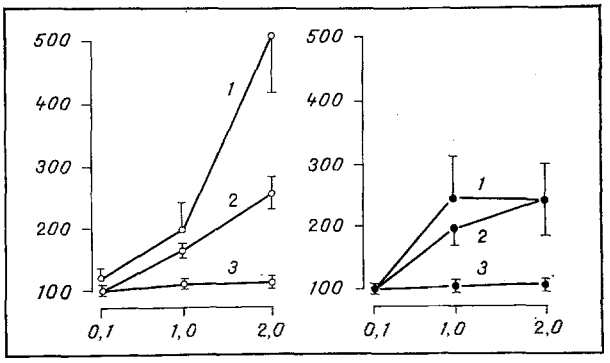

Fig. 1. Potentiation of developed tension by rest during stimulation at different frequencies and in different external Ca^{++} concentration. $CaCl_2$ concentration in solution: 1) 0.3, 2) 1.25, and 3) 5 mM. Abscissa, frequency of stimulation (in Hz); ordinate, potentiation effect (in percent). Empty circles — potentiation in myocardium of adult rats, filled circles — the same in old rats.

Ca^{++} transport are possibly the main cause of disturbances of contractility and of the force-frequency dependence in heart muscle during aging.

The effect of external Ca^{++} on chronoinotropic relations in the myocardium of adult and old rats was studied in the investigation described below.

EXPERIMENTAL METHOD

Experiments were carried out on papillary muscles of adult (aged 10 months) and old (27 months) noninbred male albino rats. The animals were anesthetized (hexobarbital, 80 mg/kg) and the heart quickly removed and perfused by Langendorff's method with Krebs–Henseleit solution (in mM): NaCl 120, KCl 4.8, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.25, $CaCl_2$ 1.25, glucose 8.6; 20°C. The posterior papillary muscle was excised from the contracting heart and placed in a chamber through which a solution of the same composition (30 ± 0.5°C) followed continuously.

The solution was saturated with a mixture of 95% O_2 and 5% CO_2, and its pH was maintained at 7.4 ± 0.02. The mechanogram and its first derivative were recorded by means of a 6MKh-2B mechanical-electronic transducer, a UZ-1 precision amplifier, a DÉ-1 differentiator, and an N338-6P high-speed automatic writer. The physiological characteristics were recorded at L_{max}. The preparations were stimulated with pulses of current of above-threshold voltage, and with a duration of 10 msec and a frequency of 0.1, 1, and 2 Hz, generated by an ÉSU-2 electrical stimulator. Before the beginning of the experimental procedures the muscle stabilized its rhythm and contracted with a frequency of 0.1 Hz for 1 h. After running in, rhythmic contractions were recorded at each frequency. Stimulation was then interrupted for 30 sec (the rest interval), after which the test contractions were recorded. The effect of the rest interval on myocardial contractility was assessed by the ratio of amplitude of the test contraction to the amplitude of rhythmic contraction. Experiments in accordance with the above scheme were carried out with different external Ca^{++} concentrations (0.3, 1.25, and 5 mM). The tension developed by the myocardium was determined as the ratio of the force of isometric contraction to the cross-section of the uncontracted muscle. In the present investigations on animals of both age groups its value was the same (0.768 ± 0.061 in adult rats, 0.779 ± 0.046 in old rats; $P > 0.05$). The significance of differences was estimated by Student's t test.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that when the Ca^{++} concentration in the solution was 1.25 mM an increase in the frequency of stimulation from 0.1 to 2 Hz caused a decrease in the developed tension, and in the rates of its rise and fall; age differences in these parameters, discovered at a frequency of 0.1 Hz, were preserved.

Lowering the external Ca^{++} level to 0.3 mM regularly led to a negative inotropic effect, whose magnitude was the same in animals of both age groups, so that differences in the absolute values of the velocity-force indices of the biomechanics between adult and old rats were still present at the low frequency of stimulation. It must be noted, however, that perfusion with calcium-free solution caused a much greater decrease in the force of isometric contraction, and also in the velocities of its development and decline in the myocardium of old animals compared with the corresponding parameters in adult rats [7, 9] and in adult rats compared with young rats [5]. With an increase in the frequency of stimulation and a deficiency of external Ca^{++} the negative inotropic effect in the heart muscle of adult rats was greater than in old rats, and this led to disappearance of age differences in the velocity-force biomechanical parameters at frequencies of 1 and 2 Hz.

Increasing the Ca^{++} concentration in the solution (5 mM) increased the amplitude of rhythmic contraction of the papillary muscles in animals of both age groups; this effect, however, occurred only at high frequencies of stimulation and was absent at a frequency of 0.1 Hz. Under these conditions the inotropic effect of frequency was not observed in either the adult or the old myocardium. Consequently, the higher the external Ca^{++} concentration the weaker the negative chronoinotropic relationship.

Potentiation of contractions by a rest interval is known to be most marked in the myocardium with a well developed sarcoplasmic reticulum (in rats), but in heart muscle without such a sarcoplasmic reticulum (in cold-blooded animals) it is absent under physiological conditions [12, 14]. During the rest interval additional Ca^{++} enters the cell, it is accumulated by the sarcoplasmic reticulum, and correspondingly more of it is released into the sarcoplasm during a test contraction. Hence it follows that ultimately potentiation of the contractions is determined by the capacity of the calcium pool of the sarcoplasmic reticulum of the heart cell [10, 11].

It will be clear from Fig. 1 that the effect of the rest interval, in an external Ca^{++} concentration of 1.25 mM, increased with an increase in the frequency of stimulation and that its magnitude was independent of age. In a deficiency of external Ca^{++} (0.3 mM) both rhythmic and test contractions were reduced. However, the amplitude of rhythmic contractions in the myocardium of the adult animals fell much more than that of the test contractions, and this caused the potentiating effect to be maximal at a frequency of 2 Hz. This was not observed in the heart muscle of old rats: the velocity-force parameters of biomechanics of the test and rhymic contractions were reduced equally in the two age groups. The smaller value of the positive inotropic effect of the rest interval at high frequencies of stimulation [4] and in solution with a low Ca^{++} concentration in the myocardium of old animals may be connected with a decrease in the capacity of the intracellular Ca^{++} depots during aging.

In the presence of an excess of external Ca^{++} the potentiating effect in the myocardium was absent altogether, in agreement with results obtained by other workers [10]. Under these conditions the Ca^{++} concentration in the intracellular depots and its release into the sarcoplasm during each rhythmic contraction evidently approached maximal values. During the rest interval there was no further increase in the calcium pool in the cell or increase in test contractions. Absence of a potentiating effect was characteristic of the myocardium of animals of both age groups in the present investigation.

It must be emphasized that in a solution with a Ca^{++} concentration of 5 mM the amplitude not only of rhythmic contractions, but also of the test contractions was lower in the old than in the adult myocardium. This probably means that even maximal entry of Ca^{++} into the cell does not lead to the disappearance of age differences in tension developed by the myocardium, the rate of its development and decline, and the temporal parameters of contraction and relaxation.

The results are evidence that chronoinotropic relations in the myocardium weaken during aging, and one reason for this is probably a reduction in the Ca^{++}-accumulating capacity of the sarcoplasmic reticulum.

LITERATURE CITED

1. V. Ya. Izakov and V. S. Markhasin, in: Textbook of Physiology. Physiology of the Heart [in Russian], Moscow (1980), p. 186.
2. V. I. Kapel'ko and M. S. Gorina, Byull. Vses. Kardiol. Nauchn. Tsent. Akad. Med. Nauk SSSR, 7, No. 1, 26 (1984).
3. O. V. Korkushko, The Cardiovascular System and Age [in Russian], Moscow (1983).
4. L. M. Laobanok, V. V. Shylau, and A. P. Kirylyuk, Vestsi Akad. Navuk Belar.SSR, No. 5, 76 (1983).
5. F. Z. Meerson and A. I. Saulya, Kardiologiya, No. 1, 71 (1984).
6. R. S. Orlov, Fiziol. Zh. SSSR, No. 5, 707 (1979).
7. V. V. Frol'kis, V. V. Bezrukov, and V. G. Shevchuk, The Circulation and Aging [in Russian], Leningrad (1984).
8. D. F. Chebotarev, Kardiologiya, No. 12, 5 (1978).
9. V. V. Shilov, in: Abstracts of Proceedings of the 1st Congress of the Belorussian Society of Gerontologists and Geriatricians [in Russian], Minsk (1983), p. 196.
10. M. Hanafy, I. Kitzing, and E. Rumberger, Basic Res. Cardiol., 71, 469 (1976).
11. R. Kaufman, R. Bayer, T. Fürniss, et al., J. Mol. Cell. Cardiol., 6, 543 (1974).
12. J. Koch-Weser and J. Blinks, Pharmacol. Rev., 15, 601 (1963).
13. T. Meinertz, H. Nawrath, and H. Scholz, Naunyn-Schmiederberg's Arch. Pharmakol., 279, 327 (1973).
14. E. Rumberger and H. Reichel, Pflüg. Arch., 332, 206 (1972).
15. P. Siegel and H. McNeil, Can. J. Physiol. Pharmacol., 60, 33 (1982).