CHARACTERISTICS OF SPINAL REFLEXES IN EXPERIMENTAL HYPOPARATHYROIDISM

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Experiments on parathyroidectomized cats with motor disorders of different degrees of severity showed disturbance of spinal reflex activity, in the form of activation of motoneurons and the interneuron system, weakening of posttetanic potentiation, and facilitation of conduction in the reflex arc. If severe tetany was present monosynaptic responses were reduced or, in some cases, abolished completely and polysynaptic reflex discharges were potentiated. After intravenous injection of calcium chloride into the experimental animals a tendency was found for recordable reflexes to return. It is concluded that disturbance of the functional state of the spinal segmental apparatus was largely due to hypocalcemia and it may be one of the causes of tetany in hypoparathyroidism.

KEY WORDS: hypoparathyroidism; calcium; mono- and polysynaptic reflex; posttetanic potentiation; efferent output.

An important approach to the study of the pathogenesis of parathyroid tetany is to investigate the functional state of the spinal segmental system in this condition. Decima [12], who used a model of experimental hypocalcemia obtained in cats by removal of the entire thyroid-parathyroid complex, attempted to demonstrate the clinical nature of the synapse between the afferent endings of group Ia fibers and motoneurons. However, removal of the whole thyroid-parathyroid complex in animals did not enable this worker to ascribe the changes he found entirely to the specific role of the parathyroid glands, for we know [3] that thyroid hormones have an important influence on spinal reflexes. With this fact in mind, it was decided to study the character of mono- and polysynaptic reflexes in animals after selective removal of their parathyroid glands.

EXPERIMENTAL METHOD

Experiments were carried out on 24 kittens, in 14 of which hypoparathyroidism (H) was produced by surgical removal of the parathyroid glands. After the operation the animals were fed on meat. The degree of development of H was assessed from the serum Ca^{++} level determined by De Waard's method, the animal's behavior, and the state of neuromuscular excitability. Usually the animals took part in the experiments 2-13 days after the operation, when all had developed motor disorders (MD) and a low serum calcium level. Mono- and polysynaptic spinal reflexes were studied in animals with different degrees of MD and after additional intravenous injection of calcium chloride. Under urethane-chloralose anesthesia (500 and 35 mg/kg, respectively) the lower lumbar portion of the spinal cord was exposed. The anterior roots L6-S1 were divided intradurally and placed on platinum bipolar electrodes to obtain monophasic records of action potentials. Small nerve branches leading to both heads of the gastrocnemius muscle, the cutaneous nerve, and the deep branch of the tibial nerve were dissected on the ipsilateral side and divided. Next, the spinal cord was transected at the level D9-10. Throughout the experiment the animal was heated (the temperature of the body and the operation wound were maintained between 36 and 37°C). The investigations began 2-3 h after the operation when the reflexes to stimulation of the central ends of the divided nerves were stimulated by pulses of supramaximal strength and 0.3 msec in duration were stable. To study the efferent output (EO) of the spinal cord repetitive stimulation was applied to the nerve to the gastrocnemius muscle with a frequency of between 1 and 100 Hz. Posttetanic potentiation of reflexes (PTR) was studied immediately after tetanic stimulation of the muscular nerve at a frequency of 300 Hz for 15 sec. The single-channel ES-103 stimulator (San-Ei Sokki) and the ÉSU-2 instrument were used for stimulation. The recording electrodes were connected to one channel of the UBP2-03

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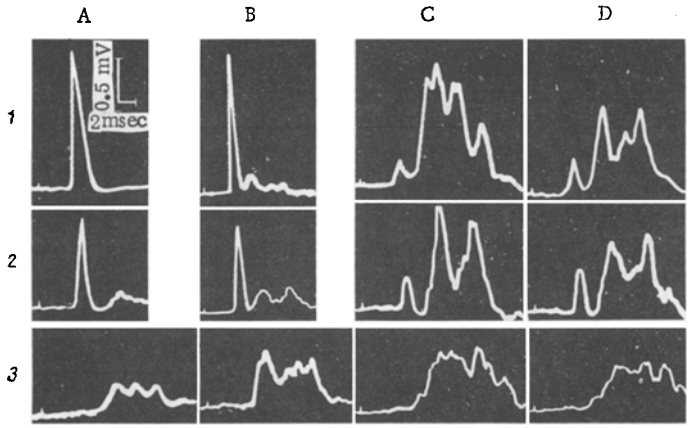

Fig. 1. Mono- and polysynaptic reflex responses in control (A) animals and in animals with mild (B) and severe (C) degrees of development of tetany and after additional intravenous injection of calcium chloride into the animals of group C (D) and stimulation of nerve to gastrocnemius (1), deep branch of tibial nerve (2), and cutaneous nerve (3) of limb.

biopotential amplifier. The output of the amplifier was connected directly to the vertical deflection plates of a type S8-11 cathode-ray oscilloscope. The statistical analysis of the results was carried out by Student's method.

EXPERIMENTAL RESULTS

Depending on the calcium level the degree of development of MD varied. In most cases, when the calcium level had fallen by 2-4 mg % rigidity of movement, unwillingness to stand up, walking with the limbs extended when pushed, and increased neuromuscular excitability were observed. When the blood calcium level had fallen by 5-6 mg % or more, the animal stood up and moved its limbs with difficulty, especially the hind limbs, developed fibrillary twitching of the muscles of the head and trunk, which in some cases gave way to spasms. The experiments showed that on the appearance of the first signs of hypoparathyroidism monosynaptic reflex responses were recorded in the anterior roots to stimulation of the nerve to the gastrocnemius muscle; the responses had a low threshold (74.0 ± 11.8 mV compared with 195.0 ± 9.7 mV in the control; $P < 0.001$), a short latent period (3.3 ± 0.13 msec compared with 4.0 ± 0.05 msec in the control; $P < 0.001$), and a shortened duration (1.8 ± 0.05 msec compared with 2.0 ± 0.01 msec in the control; $P < 0.01$). The amplitude of the responses showed no significant change. Meanwhile polysynaptic discharges were intensified and, in most experiments, they appeared even in response to stimulation of threshold (for monosynaptic responses) strength (Fig. 1B). However, the character of the change in the polysynaptic responses could be studied more accurately by stimulation of cutaneous and mixed nerves containing group II fibers. These experiments showed that polysynaptic reflexes evoked by stimulation of the cutaneous and deep branches of the mixed tibial nerve were strengthened (Fig. 1: 2B, 2C, 3B, 3C). It is interesting to note that in certain animals with a clearly defined picture of MD either total disappearance (57% of cases) or a substantial decrease in amplitude of the monosynaptic spike (43%) was observed with augmentation of polysynaptic discharges (Fig. 1: 1C, 2C). In two cats with severe manifestations of tetany (the results were not included in the statistical analysis), the reflexes studied did not appear at all. To judge the functional state of the segmental system the throughput of the EO system of the spinal cord was investigated, as other workers have done [3,5], in tetanus and thyrotoxicosis. In response to repetitive stimulation of the gastrocnemius nerve of parathyroidectomized cats by stimuli of supramaximal strength an increase in the throughput of EO was found: Rhythm binding of the monosynaptic responses was considerably improved compared with that in the control within the frequency range from 10 to 100 Hz (Fig. 2). Furthermore, in some experiments in response to repetitive stimulation of the nerve with a frequency of 3-10 Hz spasms of the muscles of the trunk and head developed. It should be emphasized that, irrespective of the degree of development of H, the direction of the changes found in the EO system of the spinal cord was the same, the only difference being that against the background of marked manifestations of tetany, when mainly the polysynaptic component could be seen, the increased output arose on account of the production of both mono- and polysynaptic components of the reflex responses.

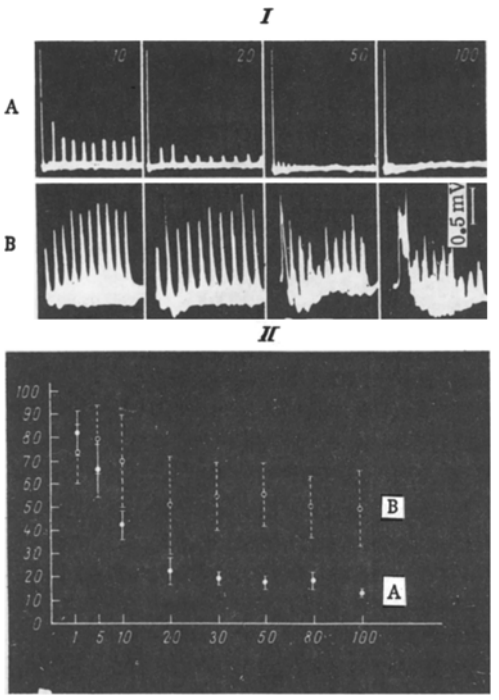

Fig. 2

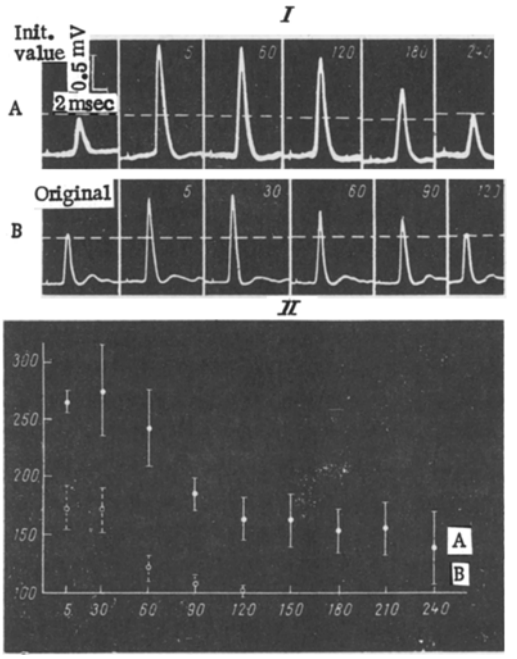

Fig. 3

Fig. 2. Throughput of efferent output of spinal cord in control (A) and parathyroidectomized (B) animals. I) Reproducibility of responses in separate experiments. Numbers indicate frequency of stimulation (in Hz); II) averaged graph (7 experiments) of reproducibility of monosynaptic responses. Abscissa, frequency of stimulation of gastrocnemius nerve (in Hz); ordinate, averaged amplitude of first 10 responses (in % of initial value).

Fig. 3. Posttetanic potentiation of monosynaptic reflex responses in control (A) and parathyroidectomized (B) animals. I) Potentiation of responses in separate experiments. Numbers denote time after end of tetanic stimulation (in sec); II) averaged graph (7 experiments) of course of posttetanic potentiation. Abscissa, time after tetanic stimulation of nerve to gastrocnemius (in sec); ordinate, amplitude of monosynaptic responses (in % of initial value).

It was natural to assume that the changes in spinal reflex activity were based on disturbances of the processes responsible for presynaptic and postsynaptic regulation of the afferent outflow. To study the state of the presynaptic apparatus in the control and experimental animals the PTR phenomenon was investigated; according to available data [14], this phenomenon is based on an increase in the liberation of mediator from presynaptic terminals. The results obtained in animals with different degrees of development of H showed substantial weakening of PTR as regards both the time of its development and its intensity (Fig. 3).

To investigate how the changes in the mono- and polysynaptic reflexes discovered during H depended on the fall in the blood calcium level in the course of the experiments some of the parathyroidectomized animals were given intravenous injections of calcium chloride. These injections raised the calcium level from 5-6 to 16-18 mg%. Stimulation (at intervals of 30 min) of the nerve against this background led to the appearance of a very slight monosynaptic response if it was previously absent or to a small increase in an existing monosynaptic and decrease in polysynaptic discharges, but only 90 min after injection of the calcium chloride (Fig. 1D). These results are in agreement with data in the literature [12] and are evidence of the calcium-dependent character of the changes observed and, in particular, of the chemical nature of the synapse between afferents of Ia fibers and motoneurons. This conclusion is also confirmed by investigations of other authors [2] who showed that some fibers terminating monosynaptically on spinal motoneurons in the frog have a chemical mechanism of transmission.

The results point to a disturbance of spinal reflex activity in animals with H. The lowering of the threshold of stimulation, shortening of the latent period and duration of the monosynaptic response, potentiation of polysynaptic discharges, and improvement in EO all suggest facilitation of excitation and conduction in the mono- and polysynaptic reflex arc. Decrease or disappearance of monosynaptic and potentiation of polysynaptic discharges in animals with infrequent manifestations of MD are evidence, on the one hand, of activation of the

system of interneurons and, on the other hand, of a decrease in the number of motoneurons involved in the reflex response. This decrease may be due to interference with the liberation of neuromediator in hypocalcemia [15] or, to some extent, to damage to the structures of the spinal arc [6], as is confirmed by the experiments with supplementary injection of calcium chloride. Under these circumstances, however, the multineuronal reflexes could be unchanged or even strengthened because of the character of activation of the motor units of the anterior horn through corresponding mono- and polysynaptic reflex chains. This explanation is supported by the potentiation of polysynaptic reflex discharges established by some workers [11] in animals after extirpation of the spinal ganglia and also in chromatolyzed motoneurons during retrograde degeneration. Similar effects have been found in the late stages of development of experimental tetanus [7] and in postischemic flexor rigidity [1]. The appearance of a polysynaptic component in response to stimulation of the nerves may also have been due to a marked increase in the excitability of neighboring fibers as a result of hypocalcemia and ephaptic transmission of excitation [10]. At the same time, potentiation of polysynaptic reflexes before any appreciable decrease in the magnitude of the monosynaptic responses may indicate weakening of inhibition in polysynaptic chains. This is indirectly confirmed by the significant decrease in the GABA concentration found by the writer [8] in the spinal cord of animals with H. In view of the important role of calcium ions in the function of the sodium–potassium pump and in ion exchange in the membranes, it is suggested [4] that in hypocalcemia due to parathyroid deficiency depolarization of the cell membranes takes place and, in the present writer's view, this may lead to increased excitability of moto- and interneurons and also to an increased throughput of the EO system. That this may be so is shown by investigations [9] in which subthreshold depolarization of the motoneuron sharply increased the frequency of discharges generated by the cell. It is also known [13] that in hypocalcemia tetanic stimulation leads to an increase in the liberation of mediator and to more marked temporal summation. This naturally can facilitate response to repetitive stimulation. The weakening of PTR which was found may be based on two mechanisms. First, it may be the result of interference with liberation of mediator by the presynaptic terminals in H and, second, it may be the result of a decrease in the subthreshold fringe of the motoneuron pool as a result of depolarization of the neurons. This would indicate weakening of the functional capacity of the spinal cord.

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