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Morphofunctional Studies of the Involvement of the Serotoninergic System in the Control of Postural and Locomotor Functions

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Various transmitter systems, including the serotoninergic, influence the locomotor behavior of animals. Studies have shown that the spinal cord, deprived of supraspinal control, has mechanisms able to induce locomotor activity in the hindlimbs and that the afferent system can trigger these mechanisms. Behavioral experiments on rats with complete transection of the spinal cord showed that pharmacological suppression of the serotoninergic system leads to suppression of motor activity associated with activation of support reactions (locomotor training). Histological studies did not identify any effect of activating support reactions on neuron survival or the distribution of synaptic contacts in spinal cord segments L2–L4. However, suppression of the serotoninergic system has been shown to lead to changes in cells in laminae 1–3 of the dorsal horns and in Rexed lamina 7, along with redistribution of synaptic contacts in Rexed laminae 1–4 in the dorsal horns of the spinal cord.

Keywords: locomotion, support reactions, spinal cord, ketanserin, quipazine.

The question of afferent control is one of the key problems in motor physiology. It is generally accepted that afferentation provides the movement control system with the information needed for constructing motor programs, that it provides feedback signals, and that it is also the mechanism of tonic activation of motor processes [1]. This latter is particularly important for the spinal motor control systems in conditions in which the connection between the spinal and supraspinal centers is broken [9] – the latter in normal conditions having the main role in initiating and regulating motor acts [14]. Studies of motor activity regulatory mechanisms at the level of the spinal cord have in recent years led to a number of important discoveries. Reported data provide evidence that the distal part of the spinal cord provides mechanisms able to induce locomotor activity in response to external influences. In animals with complete transection of the spinal cord in the lower thoracic area, stimulation of hindlimb support reactions induces locomotor movements [16, 19]. Questions related to the organization of spinal cord neural networks supporting the triggering and execution of locomotion remain open, as do those related to the role and mechanisms of involvement of neurotransmitters operating in the organization of locomotion.

Various pharmaceutical agents are known to have significant influences on locomotor activity in spinal animals. Noradrenergic preparations (for example, L-DOPA, clonidine) support the triggering of reciprocal rhythmic activity of antagonistic muscles [15, 18] and stepping motor activity in cats after complete transection of the spinal cord [8]. Agonists of the serotoninergic and glutamatergic systems do not initiate stepping movements in the absence of supraspinal control, though they can modulate locomotor activity in chronically spinal animals [6, 8, 10, 11, 17]. In rats with complete spinal

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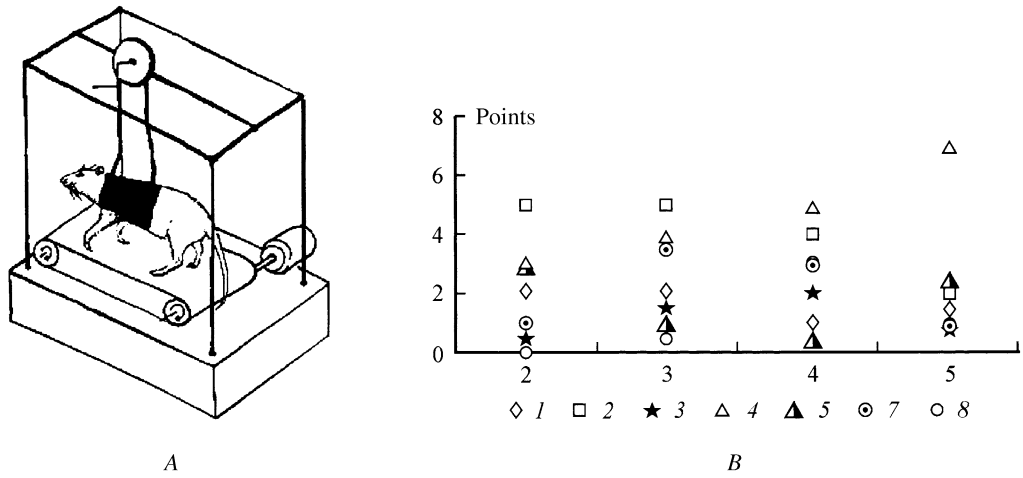

Fig. 1. *A*) Treadmill used for rat training; *B*) recovery of motor activity in rats after complete transection of the spinal cord at the lower thoracic level on the background of administration of ketanserin and different types of locomotor training. The abscissa shows weeks after transection of the spinal cord; the ordinate shows motor activity on the BBB scale. *1*, *2*, *3*) Bipedal training in combination with ketanserin administration; *4*, *5*) quadripedal training combined with ketanserin administration; *7*, *8*) ketanserin administration. Numbers correspond to the numbers of the experimental animals.

cord transection in the lower thoracic section, serotoninergic system agonists improved the characteristics of locomotion induced by epidural electrical stimulation of the spinal cord, bringing the biomechanical properties of the induced locomotion close to those of natural locomotion [12].

Recent studies showed that stimulation of the serotonin system in combination with locomotor training significantly facilitates and accelerates the recovery of motor functions in spinal animals after spinal cord injuries [11]. The authors of this latter study addressed the recovery of bipedal locomotion in rats, placing the animals in a special suspension device; a moving band ran beneath the animals' paws, providing stimulation of the supporting surface of the feet. Our studies addressed the involvement of the serotoninergic system in organizing locomotion induced by stimulation of the hindlimb supporting reactions. Studies in chronic spinal rats also demonstrated that systemic administration of the serotoninergic system agonist quipazine, on the background of training the animals on a treadmill, accelerates recovery of gait-like movements and promotes activation of the body weight support function [5]. In contrast to studies reported from Professor Edgerton's laboratory [11], we tested the locomotor characteristics in an open field using the BBB-scaling method [7]. A quadripedal gait was seen when the support was immobile and had no significant effect on movement characteristics. Histological studies of the spinal cord below the level of the transection in rats given quipazine demonstrated significant increases in the number of viable motoneurons and the number of synaptic contacts as compared with control animals not given quipazine [5]. Indirect increases in the cerebrospinal fluid serotonin level produced by electrical stimulation [2] were also accompanied in chronic spinal rats by improvements in motor functions and significant increases in mobility on testing in an open field [3].

Recent studies using an acute decerebrate cat model showed that injections of the serotonin system antagonist ketanserin prevented the occurrence of locomotor movements of the hindlimbs by epidural electrical stimulation of the lumbar bulge of the spinal cord. However, on the background of ketanserin administration and suppression of locomotor activity during epidural stimulation of the spinal cord, forced forelimb movements in cats made by the experimenter quickly triggered hindlimb stepping movements [13].

In view of these points, the aim of the present work was to undertake further analysis of the role of the serotoninergic system in the afferent control of the body weight-maintaining function and the organization of locomotion in a chronic spinal rat model. The study addressed how inhibition of the serotoninergic system (systemic administration of a serotonin receptor antagonist) influences recovery of hindlimb movements on the background of chronic stimulation of support reactions, how these movements are influenced by immobilization of the forelimbs, and how the histological features of the spinal cord below the transection level change.

METHODS

Experiments were performed of adult female Sprague–Dawley rats weighing 200–220 g. All animal manipulations were performed in compliance with the Law *On Protection of Animals from Cruelty*, Chap. IV, Article 10, 4679/11 GK of December 1, 1999." Experiments were preceded by a two-week period of acclimation of the animals. Complete transection of the spinal cord at the T9–T10 level was performed using a standard method [4]. Ketanserin (Sigma-

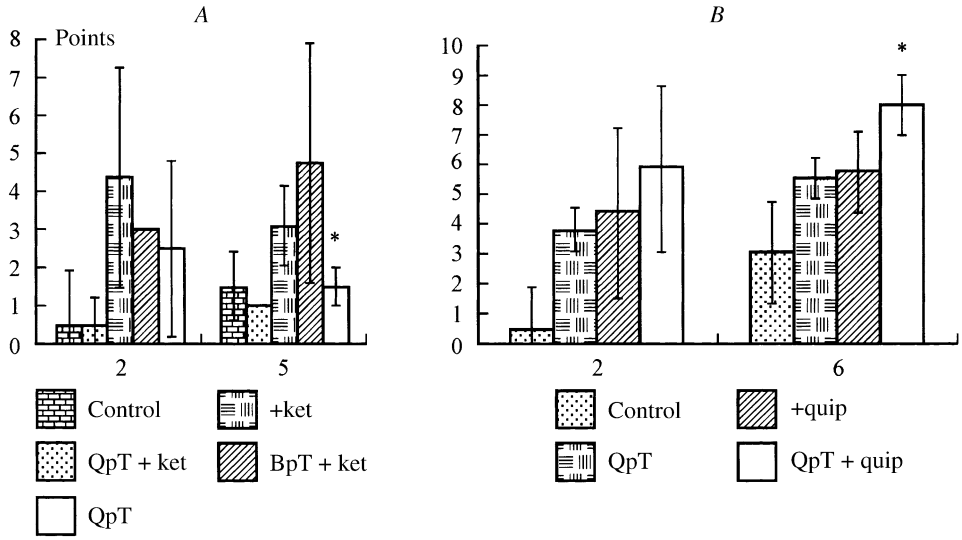

Fig. 2. Comparison of the dynamics of the recovery of motor activity in rats after complete transection of the spinal cord at the lower thoracic level. The abscissas show weeks after spinal cord transection; the ordinates show scores on the BBB scale. *A*) Effect of ketanserin administration and different types of locomotor training. Control – rats not exposed to treatments after spinal cord transection ($n = 8$); +ket – administration of ketanserin; QpT – quadripedal training on treadmill ($n = 11$); QpT + ket – quadripedal training on treadmill combined with ketanserin administration; BpT + ket – bipedal training on treadmill combined with ketanserin administration. *Significant differences ($p < 0.05$) from QpT at five weeks. *B*) Effects of quipazine administration and different types of locomotor training. Control – no treatment after spinal cord transection; +quip – quipazine administration ($n = 2$); QpT – quadripedal training on treadmill; QpT + quip – quadripedal training on treadmill combined with quipazine administration ($n = 5$). *Significant differences ($p < 0.05$) from QpT at six weeks. From [5].

Aldrich, S006) administration was started one day after surgery. Aqueous ketanserin solution at a dose of 0.2 mg/kg was given i.p. five times a week for five weeks. Training of rats on a treadmill was started at the same time (Fig. 1, *A*). During training, movements repeating the normal stepping cycle were reproduced, i.e., with plantar positioning of the hindpaws on a support. Training was also performed five times a week for five weeks, sessions lasting 15 min.

Animals were divided into three experimental groups. In the QpT + ket group ($n = 3$), quadripedal training on the treadmill was combined with ketanserin administration; the forelimbs of the rats in this group were in motion during the execution of locomotion: the rat either outpaced the running band of the treadmill or clung to the metal front bars of the suspension system, constantly sliding with it. In the BpT + ket group ($n = 3$), in which the animals were also given ketanserin, training was bipedal; the forelimbs of the animals in this group were immobilized using a tight bandage. Animals in the ket group ($n = 2$), also given ketanserin, were not trained. Controls consisted of two groups of animals, i.e., rats with complete transection of the spinal cord with quadripedal training without pharmacological intervention, and rats with complete transection of the spinal cord without training and without pharmacological intervention.

The extent of recovery of movements was assessed every week for five weeks after transection of the spinal cord using the standard BBB-scale [7]. This is a 21-point rating scale. The rat was placed in an open field and two observers independently recorded movements for 4 min, assessing parameters such as joint movements, positioning of the hindlimbs on a support, hindlimb and forelimb movement coordination, maintenance of body weight, and gait stability. The scale differentiates the extents of recovery of the support/motor capacities of the hindlimbs in rats with spinal cord injury over a wide range of trauma severities.

At five weeks after spinal cord transection, rats were anesthetized and spinal cords were fixed for histological studies by perfusion with 4% paraformaldehyde solution in phosphate buffer. Transverse sections of segments L2–L5 were stained using the Nissl method and were also studied using an immunohistochemical reaction for the synaptic vesicle marker synaptophysin.

RESULTS AND DISCUSSION

After five weeks of ketanserin administration, the maximum level of motor activity was 7 points on the BBB scale ("significant movement in three joints") (Fig. 1, *B*). This result was obtained in rats of the QpT + ket group. In rats of groups 2 and 3, subjected to bipedal training or no training, suppression of hindlimb motor activity was seen at the end of the experiments. Assessments on the BBB scale in these two groups at five weeks gave scores of 1–2, i.e., "weak movements at one or two joints" to "weak movements at one joint and significant movements at another joint."

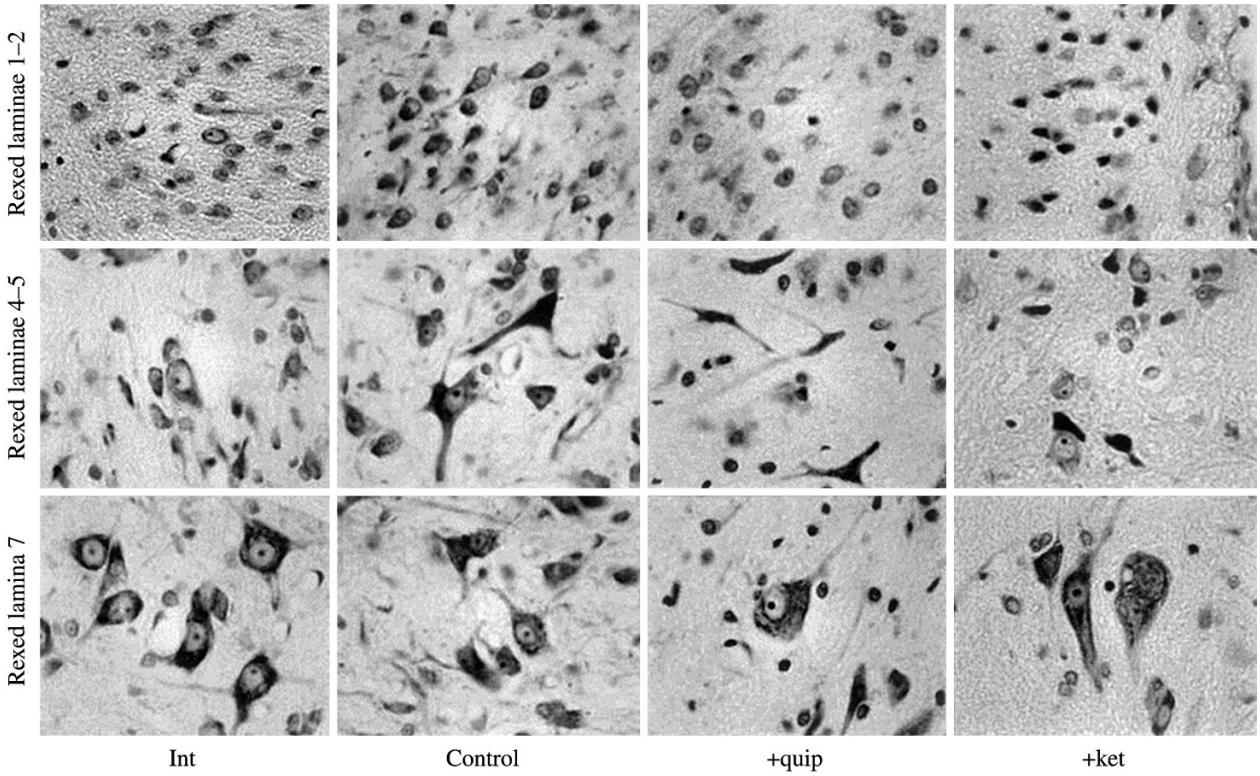

Fig. 3. Histological characteristics of the spinal cord in spinal rats after administration of an antagonist (ketanserin) and an agonist (quipazine) of serotonin receptors. Results were identical for segments L2–L4. Int – intact spinal cord; Control – complete transection of the spinal cord; +quip – spinal cord transection and administration of quipazine; +ket – spinal cord transection and administration of ketanserin. Nissl method. Magnification · 100.

Figure 2, *A* shows averaged results for all experimental and control groups. Comparison between groups of rats not trained after spinal cord transection identified minor, insignificant decreases in motor activity in the group of rats given ketanserin. Comparison between groups of rats subjected to training showed that measures of motor activity in the BpT + ket group were slightly smaller than those in in the QpT + ket group, possibly because of the high level of variation of measures in the small population of animals and the significantly smaller level of motor activity in the QpT group (rats with standard training on the treadmill with the forepaws free).

These results lead to the conclusion that inhibition of the serotoninergic system in rats with complete transection of the spinal cord prevents recovery of motor activity due to activation of support reactions. Immobilization of the forelimbs was also found to inhibit activation of the stepping movement generator by stimulation of the support reactions on the background of inhibition of the serotoninergic system. These results are consistent with previous data obtained in acute experiments in a decerebrate cat model in which inhibition of the serotoninergic system prevented the occurrence of locomotor hindlimb movements induced by electrical epidural stimulation of the lumbar bulge of the spinal cord, where forced movement of the forelimbs on the background of electrical stimulation of the spinal cord induced locomotor movements of the hindlimbs [13].

Figure 2, *B* shows results obtained by activation of support reactions in chronic spinal rats on the background of activation of the serotoninergic system by administration of the agonist quipazine (+quip). Prolonged administration of quipazine acted just as effectively as training, while the combination of quipazine and training significantly increased hindlimb motor activity on the background of activation of support reactions (QpT + quip). Assessment of motor activity on the BBB scale in some rats in this group reached 9 (“steps with support of body weight”), i.e., administration of quipazine also promoted recovery of body weight support function.

Thus, these data lead to the conclusions that the serotoninergic system has a modulatory action on the functioning of the stepping movements generator and that activation of the stepping movements generator on stimulation of support reactions is mediated by the serotoninergic system.

The results of previous histological studies of the spinal cord in rats did not identify that training had any effect on retention of spinal cord cells and the distribution of synaptophysin-immunoreactive label, this being a marker for synaptic contacts, on spinal cord cross sections. The results were

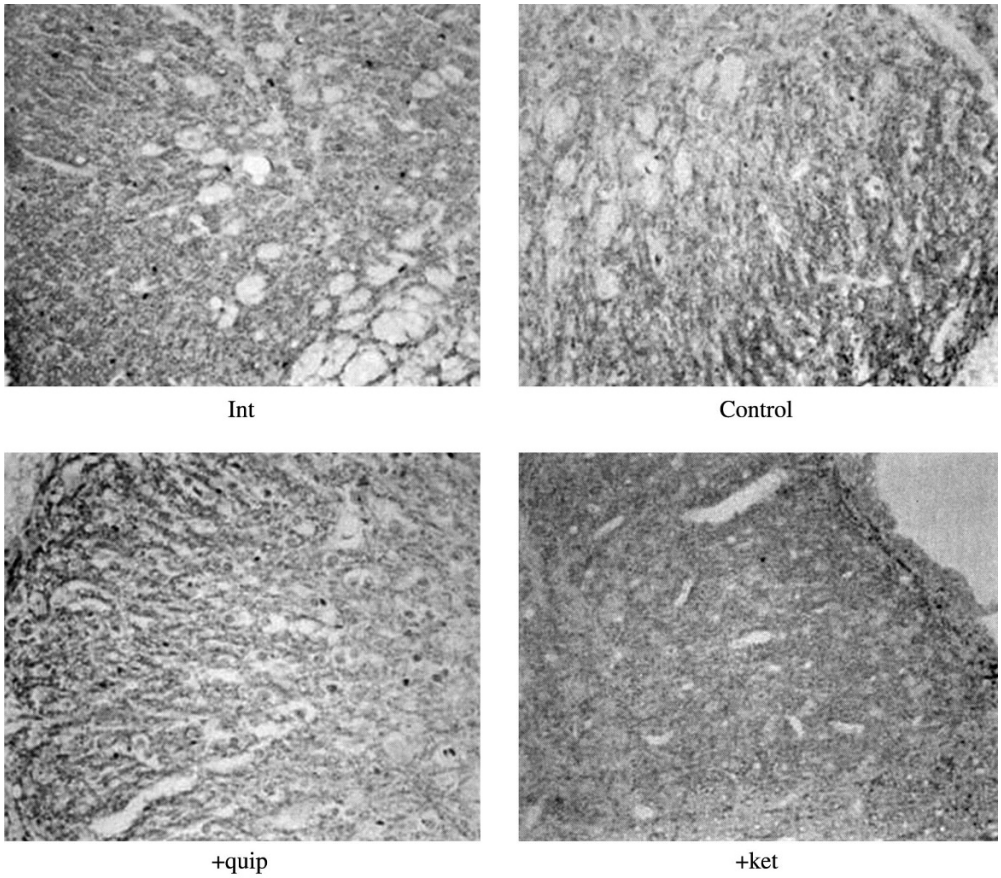

Fig. 4. Histological characteristics of the spinal cord in spinal rats after administration of an antagonist (ketanserin) and an agonist (quipazine) of serotonin receptors. Immunohistochemical reaction for synaptophysin. Results were identical for segments L2–L4. For further details see caption to Fig. 3. Magnification · 140.

therefore compared with the results of histological studies in segments L2 and L4 in rats given quipazine (a serotonin receptor agonist) and rats not given injections after spinal cord transection, as well as with the structure of intact, nontraumatized spinal cord (Fig. 3).

In normal conditions, Rexed laminae 1–3 in the dorsal horns contain small neurons. In intact control (spinal) animals and animals given quipazine, all nerve cells were morphologically preserved and there were no damaged neurons in this area. Animals given ketanserin showed significant numbers of hyperchromic nerve cells among small neurons.

In laminae 4–5 of the dorsal horns of the spinal cord, the experimental group of rats showed damage to about 30% of neurons of intermediate and small size, mainly of the hyperchromatosis type. In the control group, altered cells accounted for about 40% of all nerve cells this area, while 50–60% of neurons in the group given quipazine showed hyperchromatosis and chromatolysis.

The intermediate zone of the spinal cord – Rexed lamina 7 – in experimental animals showed numerous neurons of intermediate and large size with vacuolated cytoplasm. Such morphological pictures were not seen either in normal animals, in controls, or in experiments using quipazine. In experiments with quipazine, cells with nuclear ectopy were not seen in the intermediate zone and ventral horns. In addition, these cells were seen in trained rats (QpT + quip) and untrained rats (+quip). In the experimental group, Rexed lamina 7 also showed large neurons with central chromatolysis.

On the background of ketanserin, 30–50% of motoneurons in Rexed lamina 9 died; the remainder were morphologically intact. In all the other groups, the numbers of damaged motoneurons were greater – up to 50–60%. In lamina 9, as in lamina 7, animals of the experimental group showed vacuolization of the cytoplasm of some neurons.

In Rexed lamina 10 (not illustrated) in the dorsal commissural nucleus, the experimental group showed 50% hyperchromic (wrinkled) neurons. In the other groups, spinal animals showed similar proportions of damaged neurons (50–60% hyperchromic nerve cells in the dorsal commissural nucleus).

Immunohistochemical reactions for the synaptic vesicle marker synaptophysin showed decreased immunohisto-

chemical labeling intensity in Rexed laminae 1–4 in the dorsal horns of the spinal cord in animals of the experimental group (Fig. 4). 5-HT_2 receptors are found in cells in the superficial laminae of the dorsal horn. The decrease in synaptophysin-immunoreactive label in the neuropil of the dorsal horns appears to provide evidence of a decrease in the functional activity of neurons in this area.

Thus, exclusion of serotonin 5-HT_{2A} receptors in spinal rats in which there was no supraspinal serotoninergic innervation led to damage to cells in laminae 1–3 of the dorsal horns and some motoneurons. Neurons in lamina 7 also showed cytoplasmic vacuolization, along with marked destructive changes to neurons in lamina 10. Training in animals given ketanserin had no significant influence on the pattern of morphological changes.

It should be noted that differences in the structure of transverse sections of the spinal cord in rats given the serotoninergic system agonist were not as significant as the differences in motor functions in the animals of this group. This may be due to the fact that the histological study methods used in our experiments probably do not identify interneuronal connections, which are undoubtedly among the causes of activation and inhibition of the stepping movement generator.

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