(WGA-HRP) were injected in the caudal lumbar and rostral sacral cord, bilaterally, in order to retrogradely label spinally projecting neurons in the PMC and to anterogradely label terminals on these neurons. After a survival time of 18 hours the animals were perfused with a fixative containing 1% paraformaldehyde and 2 % glutaraldehyde in a 0.1 M phosphate buffer. The tissue was processed with standard light and electron microscopical techniques.

RESULTS

Light microscopy showed that both anterogradely and retrogradely labeled profiles were present in the PMC after WGA-HRP injections in the caudal lumbar and rostral sacral cord. Ultrastructural analysis demonstrated that many anterogradely labeled terminals were present in the area of the PMC, which were filled with round, pleiomorphic and flat vesicles. In about 15% the terminals contacted retrogradely labeled dendrites. The majority contained asymmetric synaptic clefts, but also symmetric clefts (25%) were found.

CONCLUSIONS

The results provide evidence for a direct lumbosacral-PMC pathway in the rat. This implicates that sensory neurons involved in processing bladder filling information have direct access to the PMC, which is not the case in cats. The results provide an explanation for the differences in micturition behavior between rats, and cats and humans. Thus, species dependent differences in neuronal pathways involved in micturition reflect differences in their micturition pattern.

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A RAT MODEL FOR INVESTIGATION OF BLADDER DYSFUNCTION ASSOCIATED WITH MULTIPLE SCLEROSIS-LIKE DEMYELINATING DISEASE

AIMS OF STUDY

Myelin basic protein (MBP) can be used as antigen for inducing experimental allergic encephalomyelitis (EAE). Various studies have been reported using EAE animals as an experimental model of demyelinating diseases such as multiple sclerosis. However, no study on the bladder dysfunction in EAE animals has been reported. The aim of this study was to determine whether an EAE rat induced by MBP is useful for the investigation of bladder dysfunction associated with demyelinating disease.

METHODS

Female Lewis rats (weighing 175-245 g) were used.

Study 1) Time course of behavioral and cystometric changes

MBP injection : Fifty μg (10 μl) of MBP was injected subcutaneously in the right hindpad of 13 rats and saline as the vehicle in 5 control rats.

Behavioral investigations : Behavioral changes were investigated at least once a day during for 21 consecutive days after injection of MBP or saline.

Cystometric investigations : A polyethylene catheter was implanted into the bladder through the dome 3 days after injection. Cystometric investigations were performed without any anesthesia for 12 consecutive days from 7 days after injection. Intravesical pressure and micturition volumes were recorded continuously on a polygraph. Detrusor hyperactivity (DH) was defined by the following criteria: micturition volume less than 0.3 ml and residual volume less than 0.1 ml.

Study 2) Correlation between behavioral, cystometric and histological abnormalities

MBP injection : Separately from the Study 1, 50 μg of MBP was given to 20 other rats in the same manner as described above.

On the basis of the results of Study 1 described below, behavioral, cystometric and histological abnormalities were investigated on the day 12.

Behavioral investigations : The degree of paralysis was classified : grade 0 ; no paralysis, grade 1 ; paralysis only of the tail, grade 2 ; incomplete paralysis of the hind limbs, grade 3 ; complete paralysis of the hind limbs.

Cystometric investigations : Bladder catheter implantation was made on the day 9. Continuous cystometry was performed without any anesthesia.

Histological investigation : Immediately after behavioral and cystometric investigations, the rats were perfused transcardially with 80 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) under general anesthesia, and the brain and spinal cord were removed, then kept overnight in the same fixation solution at room temperature. Investigations were performed mainly of the spinal cord at the level of L6-S1. The brain, and the spinal cord levels C5, Th4 and L2 were also investigated in some animals. Histological findings were evaluated with a double blind method by two investigators, and inflammation observed was scored as the following three degrees : Mild (1) ; no parenchymal infiltration / meningeal mononuclear cells infiltration, Moderate (2) ; mononuclear cells almost localized to focal area of neuronal parenchyma and Severe (3) ; panmyelitis involving both white matter and gray matter. The inflammation score was defined as the sum of the points (from 1 to 3) of the two investigators.

Wilcoxon signed rank test was used for statistical analysis.

RESULTS

Study 1) Time course of behavioral and cystometric changes

Behavioral investigations : Hind paralysis was observed in all the rats but one given MBP. The onset of the hind paralysis was at the day 10-14 (mean 12.2) after MBP injection, and the duration of the paralysis was for 1-7 (mean 3.5) days. Then all rats recovered and looked normal.

Cystometric investigations : In the MBP-injected group, the cystometric findings were characterized by three different patterns : 1) Detrusor areflexia / Detrusor hypoactivity (DA) ; 2) DH, and 3) Normal.
Ten (77%) of the 13 rats given MBP showed bladder dysfunctions including 7 DA, 2 DA/DH and 1 DH (Fig. 1). All these bladder dysfunctions were transient and restored to normal by the day 18. All the 5 rats given the vehicle showed normal pattern and their mean micturition volume was 0.44 ml.

Study 2) Correlation between behavioral, cystometric and histological abnormalities

Cystometric investigations showed DA in 10, DH in 1, and normal findings in 9 animals. All the rats with DA showed grade 3 paralysis, whereas among the 9 rats that were cystometrically normal, 1 showed grade 0, 3 grade 1, and 5 grade 2 paralysis. The mean paralysis grade in the DA group was 3.0, whereas the corresponding value was 1.4 in the cystometrically normal group. The difference between the two groups was statistically significant ($p < 0.01$). The mean value of the inflammation score at the level of L6-S1 spinal cord in the DA group was 5.2 ± 0.9, which was significantly ($p < 0.01$) higher than that in the cystometrically normal group (3.3 ± 1.1). The corresponding value in the rat with DH was 2.0. The scores in the brain, and at spinal cord levels C5, Th4, and L2, were much less than at the L6-S1 levels. The degree of paralysis was significantly correlated ($\gamma = 0.563$, $p < 0.01$) with the inflammation score at the L6-S1 spinal cord.

CONCLUSIONS

The present results suggest that MBP can induce bladder dysfunction (DA/DH) in rats. The bladder dysfunction is transient and reversible, occurring concomitantly with hind limb paralysis and inflammatory changes at L6-S1 spinal cord level. Thus, the present rat model seems useful for studies of bladder dysfunction associated with spinal myelitis / demyelinating diseases.

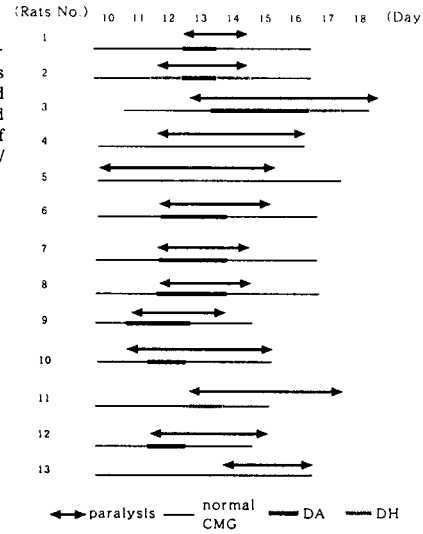

Fig. 1

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SACRAL ROOT NEUROMODULATION INDUCES INHIBITION OF THE HYPERACTIVE C-AFFERENT FIBERS IN A HYPERREFLEXIC ANIMAL MODEL

INTRODUCTION: Sacral root neuromodulation is an impressive new concept that has been used recently for treatment of refractory voiding and storage problems. Unfortunately, the mechanism of action remains to be clarified. We hypothesize that sacral root neuromodulation works through inhibition of the C-afferent fibers. According to the gate theory of Melzack and Wall [1] stimulation of the afferent fibers that is converging on the same dermatome of a smaller size afferents can inhibit the later fibers. Several reports have shown that bladder activity can be inhibited by stimulation of afferents from different sources that converge on the same dermatome of bladder innervation. (2,3,4)

METHODS: Sixty female Sprague Dawley rats have been included in this study. These have been divided into three groups of normal controls, spinalized rats at T10 and spinalized rats that have been subjected to bilateral S1 electrostimulation for 6 hours daily. Three weeks post spinalization, urodynamics was performed and Substance P (SP), Neurokinin A (NKA) and Calcitonin gene related peptides (CGRP) were extracted from the dorsal root ganglia (DRG) of L5 and L6 roots and quantified using radio immuno-assay (RIA). In addition electrophysiological studies for the reflex arcs supplying the urinary bladder