Short communication

Pentobarbital-induced modulation of flexor and H-reflexes in Spinal rats

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Accepted 8 August 2000

Abstract

Electrophysiological recordings of the H-reflex and nonnociceptive flexion reflex were obtained from pentobarbital-anesthetized Intact rats and from both, anesthetized and unanesthetized groups of Acute and Chronic Spinal rats. Results showed that the flexor, but not H-reflex, of Chronic Spinal rats was significantly larger than that of all other groups, which did not differ among themselves. The antispastic drug baclofen dose-dependently decreased the flexion response of Chronic Spinal rats (A4.3 mg/kg; 2.1 and 9.0 mg/kg).© 2000 Elsevier Science B.V. All rights reserved.

Theme: Motor systems and sensorimotor integration

Keywords: Spasticity; Spinal rat; Flexor reflex; H-reflex; Rate dependent suppression; Baclofen

Spinal cord injury (SCI) produces devastating functional losses, including spastic paresis and chronic pain, for which present treatments are often unsatisfactory [3,20]. Efforts to improve available therapies include the development of animal models that approximate at least some aspects of the clinical conditions [15,18].

Most of these studies use at least one of two electrophysiological measures to assess reflex function, i.e. the Hoffman (H)-reflex or the flexor reflex (FR). The H-reflex is the electrophysiological expression of the monosynaptic (stretch) reflex [11,14,15,19] and exaggeration of this response may occur in SCI as one manifestation of spasticity [12,17]. When elicited at frequencies greater than 0.3 Hz, there is a decrease in H-reflex amplitude in normal humans, termed frequency, rate-dependent or post-activation depression, which is reportedly reduced in spastic human patients [4,8,12,17]. The FR is a polysynaptic response evoked in the flexor muscles, usually of the hindlimb, by either nociceptive (e.g. ≥6.8±0.2 mA of shock; 6) or nonnociceptive input. Because this reflex may also be hyperreactive in spasticity [13], the FR has been used to evaluate the effects of centrally acting muscle relaxants in nonhuman animal models, produced by nonnociceptive stimulation [11,14,16,19]

Unfortunately, in animal models, these electrophysiological recordings are usually invasive and often require the use of anesthetics, which may mask or interact with the physiological condition or assessment of drug action. For this reason, we have recently developed the Chronic Spinal rat as an experimental model in which to assess electrophysiological indices of reflex function in the awake, unanesthetized animal [1,2]. Experiment 1 extends our previous work, in two ways. First, parameters of the H- and flexor-reflex, both of which were obtained from each rat, were compared in Intact, Acute Spinal and Chronic Spinal groups for the development of hyperreflexia after spinal transection. Second, because the Intact rats had to be anesthetized for these recordings, we included additional groups of Acute and Chronic Spinal rats, which also received the anesthetic. Pentobarbital [14] was chosen because our preliminary studies indicated that volatile anesthetics (e.g. isoflurane; 5) abolished the nonnociceptive FR (unpublished data); ketamine [11,15,18] had an excitatory effect on the FR in intact rats (unpublished data); and an α-chloralose/urethane combination [11,14,19] can only be used if the preparation is terminal.

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which would preclude using the Chronic Spinal rat for evaluation of multiple agents or chronic drug administration.

In Experiment 2 we assessed the effect of (±) Baclofen (BAC; a GABAₐ agonist) on the nonnociceptive FR of chronic spinal rats. Although baclofen is presently the most efficacious, clinically available agent for the treatment of spasticity [7,10], most of the nonhuman behavioral research has assessed its purported analgesic effects. To our knowledge this is the first report describing the dose-related effect of systemic baclofen on the nonnociceptive FR in a proposed animal model of SCI-induced spasticity.

Experiment 1 included 33 male albino Sprague–Dawley rats (Division of Laboratory Animal Medicine, Louisiana State University Veterinary School, Baton Rouge, LA), weighing an average of 348 (±2) g; and Experiment 2 used 28 male albino Sprague–Dawley rats (Holtzman Laboratories, Madison, WI), weighing an average of 339 (±6) g. All rats were singly housed in plastic cages in a colony room maintained on a 12:12 h light:dark cycle, with dark onset at 19:00 h, and had continuous access to food and water. The procedures for spinalization and post-operative care have been described in detail [1,2]. Acute Spinal rats (Experiment 1) were tested 2 days after surgery and Chronic Spinal rats were tested an average of 41 (±<1) days (Experiment 1) or 35 (±1) days (Experiment 2) after surgery. All stimulation and recording procedures were performed with a Nicolet Viking IV D system (Nicolet Instrument Corporation, Madison WI). At the end of the experiment the rats were euthanized by an anesthetic overdose or administration of CO₂. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Louisiana State University (Baton Rouge, LA).

In Experiment 1, five groups of rats were injected intraperitoneally with either 50 mg/kg pentobarbital or saline (1 ml/kg). There was one group of unoperated, Intact rats (Intact/Pento: n=7), two groups of Acute Spinal rats (Acute/Pento: n=7; Acute Spinal: n=7), and two groups of Chronic Spinal rats (Chronic/Pento: n=6; Chronic Spinal: n=6). Thirty minutes after the respective injections, the tibial nerve was stimulated (single square-wave pulses; 0.2 ms duration) using a pair of stainless steel barbed broaches (Patterson Dental, New Orleans, LA) inserted percutaneously into the plantar muscles.

Prior to making the experimental recordings, 32 successive stimulations of increasing intensity (0.8–26.7 mA) were administered to determine the stimulus required to elicit the maximum reflex amplitude for each rat. Magnitudes of the evoked EMG potentials were obtained by measuring the peak-to-peak amplitude (mV) of each H-reflex, as well as the preceding M-wave (produced by direct stimulation of motor nerve fibers).

To examine rate-sensitive depression, the stimulus intensity that produced the maximum response was used to elicit 10 consecutive reflexes at each of four frequencies: 0.3, 1.0, 3.0 and 5.0 Hz, with a 5-min inter-rate interval. Both, H-reflex response amplitudes and latencies (ms) were obtained for all 10 responses at each stimulus rate and the means determined for each frequency.

Following H-reflex assessment, the FR was examined in the contralateral leg, using the same procedures as previously reported [1,2], i.e. five square wave shocks, at 500 Hz, 0.2-ms duration. Stimulus intensity was set at 2.5× threshold and five responses were elicited at approximately 30-s intervals. Each response was rectified and integrated, within a time window of 200 ms, providing an index of the area under the curve (AUC) in mV×ms as the measure of reflex magnitude. The FR response was defined as the mean of the five elicited FR responses for each rat.

For comparisons among the groups, the M-wave, H-reflex and FR responses were analyzed by either one-way ANOVAs or two-way repeated measure ANOVAs (Sigma-Stat, Jandel, San Rafael, CA). If a significant overall effect was indicated, post-hoc tests (Newman–Keuls) were performed to determine which groups differed.

In Experiment 2, the effect of baclofen on the FR of Chronic Spinal rats was tested by first obtaining five pre-drug baseline scores for each rat. Separate groups were then injected with either saline (n=9) or subcutaneously with 1.0 (n=4), 3.0 (n=5), 10.0 (n=5), or 30.0 (n=5) mg/kg (±) BAC (Research Biochemicals, Natick, MA). The FR was again assessed 30, 60, 90 and 120 min later. At each post-drug test point, the effect of BAC was quantified as percent (%) of baseline with the formula:

\[
\text{post-drug score/pre-drug score} \times 100
\]

The area under each time-effect curve (AUC) was calculated for each rat with the computer program PHARM/PCS (Microcomputer Specialists, Philadelphia, PA). With this transformation a dose–response curve was obtained and the A₉₀ value (±95% Confidence Intervals; CI) was calculated using the Litchfield–Wilcoxon method (PHARM/PCS, Microcomputer Specialists). This dose–response function was also analyzed by one-way ANOVA. For all statistics, results were considered significant at \( P<0.05 \).

Table 1 summarizes the stimulus intensities and corresponding response magnitudes for the FR, and the maximum H- and M-waves. The mean shock intensity for elicitation of the FR (2.5×threshold) differed among the groups \( F(4,32)=4.61, P<0.005 \). Specifically, the mean shock value for the Chronic Spinal rats was significantly lower than that of all pentobarbital groups (i.e. all groups except the Acute Spinal group). The magnitude of the FR also differed among the groups \( F(4,32)=11.91, P<0.001 \). Importantly, the Chronic Spinal rats had a significantly larger FR (in response to a lower threshold stimulus) than all other groups.

Although the stimulus (mA) necessary to produce the maximum H-reflex did not differ among the groups, there was a difference in amplitude (mV). This reflex was...
significantly larger in Chronic Spinal rats than Intact/Pento rats \([F(4,32)=4.19, P<0.01]\). There was no significant difference across conditions in the stimulus necessary to produce the maximum M-reflex (data not shown), or its amplitude.

As seen in Fig. 1A, rate-dependent depression was obtained, in that the amplitude of the H-reflex, across all frequencies, differed among the groups \([F(4,84)=8.91, P<0.001]\). Overall, the H-reflex of Chronic Spinal rats was significantly larger, i.e. rate-sensitive depression was less, than the response of all pentobarbital groups (i.e. all except the Acute Spinal group). In addition, rate-sensitive depression in Acute Spinal rats was less than in Intact/Pento and Acute/Pento rats. Taken together, these results suggest that pentobarbital increases rate sensitive depression of the spinally transected rat.

As seen in Fig. 1B, there were also significant differences in H-reflex latencies. First, collapsed across frequency, there was a main effect of condition \([F(4,84)=9.97, P<0.001]\), in that, the latency of the Acute/Pento group was longer than all other groups and the latency of the Chronic/Pento group was longer than that of the Chronic Spinal group. These results show that pentobarbital increases the H-reflex latency of the spinally transected rat. Second, there was a main effect of frequency, i.e. H-reflex latencies became longer at each of the stimulus frequencies \([F(3, 84)=27.83, P<0.001]\). Third, there was a significant interaction between condition and frequency \([F(12, 84)=4.05, P<0.001]\). These results suggest that, regardless of condition (i.e. intact or spinally transected) pentobarbital increased the latency of the H-reflex.

Fig. 2 shows the effects of BAC on the FR of Chronic Spinal rats. As expected, BAC produced a dose-dependant decrease in the magnitude of the FR \([F(4,27)=10.32, P<0.001]\) with an \(A_{50}\) of 4.3 mg/kg (95% CI: 2.1 and 9.1). Though they did not differ from one another, the two highest doses (10 and 30 mg/kg) significantly reduced the FR in comparison to all other groups.

These results (Table 1) show that both, spinalization and pentobarbital, significantly and differentially affected reflex function. The stimulus intensity necessary to evoke the FR in Chronic Spinal rats was lower than that required for the three pentobarbital-treated groups, which did not differ among themselves. The fact that the stimulus intensity under pentobarbital was the same for Intact, Acute and Chronic Spinal conditions suggests that the drug (at the 50 mg/kg dose) was more potent in Chronic than in Acute Spinal rats.

This differential effect is more evident in regard to response magnitude, which was substantially greater in the Chronic Spinal rats than in all other groups, which did not differ among themselves. Again, the size of the FR in Acute Spinal rats under pentobarbital was the same as that of Acute rats without the anesthetic. In contrast, the response of Chronic Spinal rats was larger without the anesthetic. This outcome is consistent with an earlier report of increased FR ‘sensitivity’ of Chronic (2 months) Spinal compared with Acute (40–48 h) Spinal rats, to intraperitoneal administration of a variety of drugs [9]. Taken together, the decrease in stimulus intensity required to elicit the flexor, and the hyperresponsiveness of the Chronic Spinal rats, support the development of spasticity in this preparation, although, admittedly, this conclusion must be tentative because comparisons could not be made with an unanesthetized, Intact group.

Unlike the flexor, there was no difference between Acute and Chronic Spinal rats in either intensity or amplitude of the maximum H-reflex, or M-wave. Nor did pentobarbital differentially affect these measures in Spinal rats. However, pentobarbital did significantly influence rate-dependent suppression of the H-reflex. It decreased amplitudes and increased latencies compared to the response of the respective, unanesthetized, spinal groups. But Acute and Chronic Spinal rats did not differ in the absence of pentobarbital. Furthermore, the latencies of unanesthetized Spinal rats did not differ across stimulus frequency, whereas the latencies of pentobarbital-treated rats increased as a function of frequency. Finally, the pentobarbital-induced increase in latency was significantly greater in Acute Spinal than Chronic Spinal or Intact rats, suggesting that the Acute Spinal rats were more vulnerable to the anesthetic.

The absence of a hyperactive H-reflex, or impaired rate-dependent inhibition in Chronic, relative to Acute Spinal rats, does not support the use of this measure as a model of SCI-induced spasticity. Yet, this conclusion might
The data represent the AUC / 10 (±S.E.M.) of the values obtained at 30, 60, 90, and 120 min after SC injection of 1.0, 3.0, 10.0, 30.0 mg/kg BAC, or saline administration. There was a significant difference among the groups. * At doses of 10.0 and 30.0 mg/kg BAC significantly reduced the flexor reflex compared to all other doses and saline (\( P < 0.05 \)). The \( A_{\text{up}} \) was 4.3 mg/kg (95% CI: 2.1 and 9.1).

Although this H-reflex analysis did not provide evidence of spasticity, there was a significant influence of pentobarbital, which was consistent with recent results showing that halothane anesthesia also increased the stimulus threshold of the H-reflex in Acute Spinal rats, relative to an unanesthetized group [5]. Because Spinal animals do not require anesthesia, they may provide useful comparisons with results from clinical investigations of awake, human subjects. In fact, one recent study [13] reported that 88% of spastic patients had ‘pathologically enhanced flexor reflexes in the lower limbs,’ . . . ‘accompanied in 47% of cases by abnormal decrease of reflex threshold.’ Administration of intrathecal baclofen reduced amplitudes and increased thresholds in all patients. It was concluded that data from FR recordings added to that provided by clinical measures, and that the FR was ‘a useful tool’ for ‘quantifying the benefit of antispastic treatment.’

Acknowledgements

The authors would like to thank Michal Maranto for her assistance in performing the surgical procedures and data entry.

References


