Effects of Different Anesthetics on the Paired-Pulse Depression of the H Reflex in Adult Rat

Stephen M. Ho*† and Phil M. E. Wait†

*Developmental Neurobiology, Research School of Biological Sciences, Australian National University, Canberra, ACT 2601, Australia; and †Neural Injury Research Unit, School of Medical Sciences, University of New South Wales, Sydney, New South Wales, 2052, Australia

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Hyperreflexia is a common feature of spinal cord injury (SCI), and changes in reflex excitability have been reported to be useful in assessing treatments in animal models of cord damage. However, spinal reflexes are known to be dependent on anesthetic level. As a preliminary to its use in SCI, the excitability of the Hoffman reflex (H reflex) has been assessed under several commonly used anesthetics. The H reflex was recorded in the distal foot muscles (dorsal interossei) of adult rats, while the lateral plantar nerve was stimulated. Five different anesthetics were used: ketamine, halothane, Nembutal, Etomidate, and Saffan. Recording and stimulating electrodes were inserted directly through the skin to minimize the surgical procedure for each experiment, allowing repeated recording to be made in the same animal on a weekly basis. Suppression of the H reflex was tested using twin-pulse stimulation. Halothane and ketamine produced suppression of the H response when interpulse intervals were shortened to less than 1 s. The H-reflex suppression profiles recorded under Etomidate, Saffan, and Nembutal anesthesia were less sensitive to the stimulation rate, with little reduction until intervals were 200 ms or less. The suppression profiles of halothane and ketamine resemble that seen in unanesthetized animals, whereas that under the other anesthetics tried here resembles that observed in spinal-cord-injured animals. The results suggest a preferential action of some anesthetics on descending pathways involved in reflex modulation and the importance of assessing reflex excitability under anesthetics such as ketamine or halothane.

Key Words: general anesthesia; monosynaptic reflex; spasticity; rate depression; spinal cord injury; hyperreflexia.

INTRODUCTION

The Hoffman reflex (H reflex) is the electrical analogue of the tendon jerk reflex, a monosynaptic reflex mediated through the spinal cord. Electromyograms (EMG) recorded during an H reflex typically show two responses: an initial M wave and a later H wave. The M wave is the result of the direct activation of the motor axons while the later H wave is the EMG elicited by the synaptic activation of motoneurons by group Ia muscle afferents through the spinal reflex pathway. In the normal situation, the magnitude of the H wave is subject to attenuation by repetitive activation (7, 25, 37). This is thought to be due to presynaptic mechanisms and to be dependent on descending controls (11, 21, 30). Such rate-sensitive depression has therefore been found to be useful for assessing the integrity of descending controls. After spinal cord injury, this rate-sensitive depression of the H reflex is progressively abolished, accompanied by the development of hyperreflexia and spasticity (18, 37). Recent studies suggest that this increase in reflex activity is not always permanent but can be reversed in some animal models of spinal injury. For example by imposing an exercise regime or using fetal spinal cord transplants, Skinner et al. reported that the rate-dependent suppression of H reflex was improved toward control values (33). An improvement in locomotor behavioral score, and in the rate resistance of the H reflex in the control situation, was also reported after using olfactory ensheathing cell therapy after transection injuries (23). It appears, therefore, that the rate-dependent depression of the H reflex is useful for determining chronic changes in spinal cord functions after spinal injury. This is especially important during regeneration studies in which the direct action of regenerated fibers may become diffused and difficult to detect. Since the recording of the H reflex requires minimal surgical interventions, repeated recordings at regular intervals can be made in the same animal, providing an ongoing assessment of spinal cord functions for determining the effectiveness of certain therapeutic protocols.

As it is necessary to stabilize the animal and minimize voluntary activity during H-reflex recordings, tranquilizers or general anesthetics are commonly used. A common dilemma for in vivo electrophysiolog-
Anesthetic studies is the potential effect of anesthetics on the neural pathway that is being studied, in this case the spinal reflex and its modulation by descending controls. In the present study we used a recording method which involved minimal surgical procedures, in which both stimulating and recording electrodes were inserted through the skin without any incision, so that the experiments could be repeated in the same animal with different anesthetics. We tested several common general anesthetics with different mechanisms of action and compared their effects on the H reflex, especially the property of rate-dependent depression. We were particularly interested to compare the anesthetic ketamine, commonly used for recording the H reflex in animal models, with other frequently used anesthetics such as halothane and Nembutal. We found that halothane, with a narrow dose margin, produced a rate-dependent suppression of the H reflex similar to that of ketamine. However, several anesthetics produced rather unexpected results. The H-reflex rate depression profile observed under Etomidate, Saffan, and Nembutal showed a rate-resistant profile, similar to those described in animals with spinal cord injury.

MATERIALS AND METHODS

Animals

Experiments were conducted on adult albino (Wistar) rats in the weight range 180–250 g. All procedures were in accordance with the Australian National University Experimentation Ethics procedure. All recordings were made while the animals were anesthetized with one of the general anesthetics: ketamine (Troy Laboratories, Australia; n = 10), halothane (Laser Animal Health, Australia; n = 6), Nembutal (pentobarbitone sodium, Rhone Merieux, Australia; n = 6), Etomidate (Janssen Pharmaceutica, Belgium; n = 4), and Saffan (alpha-xalone/alpha-dolone; Pitman-Moore Australia Ltd.; n = 4). Halothane was delivered through a nose cone using compressed air at a flow rate of 10 L/min and tested in the range of 0.5–1.5%. Ketamine was administered at a dosage of 120 mg/kg ip and animals were pretreated or co-injected with xylazine (2 mg/kg) as a sedative for the first dose only. Other initial anesthetic doses were as follows: Etomidate 20 mg/kg ip, Saffan 80 mg/kg im in two equal doses, Nembutal 30 mg/kg ip. Vital signs for the animal, including heart rate, respiration rate, core body temperature, and reflex excitability (paw pinch and corneal touch), were monitored and recorded every 15 min. Additional anesthetic was administered as necessary to just suppress withdrawal reflexes to paw pinch. Core body temperature was measured with a rectal probe and maintained at 37 ± 1°C with a heating pad. ECG recording was used to monitor the heart rate. The preparation was grounded through the ECG amplifier. After each recording session, the animal was placed in a warmed boxed until fully recovered from the anesthetic before being returned to its normal caging. Each animal was subjected to repeated recording at weekly intervals for 6 weeks and different anesthetics were used in subsequent experiments.

H-Reflex Recording

Surgical procedures for each recording session were kept to a minimum to allow repetitive recordings to be made in the same animal at a weekly intervals. While anesthetized, the left hind limb was secured with sticky tape (Sellotape) onto a platform. The skin at the recording and stimulation sites was disinfected with Hibitane (0.5% in 70% alcohol). The H reflex was evoked by stimulating the lateral plantar nerve and recorded in the hind-foot interosseous muscles. The nerve was stimulated with a pair of sharp tungsten bipolar electrodes inserted directly through the skin on the lateral side of the ankle. The position of the stimulating electrode was adjusted to maximize evoked movements in the digits following stimulation. Likewise EMG recording was made from the interosseous muscles between the fourth and the fifth or the first and the second metatarsals with sharp tungsten bipolar electrodes (exposed tip about 500 μm) inserted through the skin without any incision. EMG responses were recorded with an A/C coupled differential amplifier and stored on a computer using the Axotape software (Axon Instruments, CA) for later analysis. Square stimulating pulses of 0.1 ms duration were used at a rate of 1 per 20 s and the stimulation intensity was adjusted to give a steady M wave. This was between 1.2 × threshold (T) and 3 T for most experiments. Using such criteria, the H response may differ in amplitude between preparations but will not affect the rate-depression properties (33). To determine the paired-pulse depression of the H reflex, twin-pulse stimulation with conditioning and test pulses was used at various interpulse intervals between 5 s and 30 ms. A total of 10 sweeps were recorded at each interval. The M and H waves were quantified by rectifying the waveform and integrating the area bound by the response curve for 3 ms. The baseline was defined by the prestimulation level. Digital integration was carried out using a computer program developed with the Matlab software package (The Math Works, Inc., MA).

Spinal Cord Contusion Injury

To compare the rate depression of the H reflex in spinal-cord-injured animals, spinal cord contusion was induced in three animals using the New York University Spinal Cord Contusion System (Impactor) developed by Drs. John Gruner and Wise Young (15). The animal was anesthetized with Nembutal (60 mg/kg) and xylazine (2 mg/kg) ip. Under aseptic conditions a
dorsal laminectomy was performed at the T12 vertebral level to expose the cord at L1/L2 and spinal clamps were attached at T10 and L1. Bupivicaine was infiltrated around the cord at the impaction site and dripped onto the bone edges to provide analgesia of the periosteum. A drop height of 50 mm was used to produce a severe contusion from which recovery of locomotor activity was minimal (6). The wound was closed in layers and the skin sutured. The animal was kept in a warmed box and inspected every 30 min until fully conscious. Postoperative care included fluids and a nonsteroidal anti-inflammatory analgesic, Carprofen (5 mg/kg/day, subcutaneous), for the first 3 days after surgery. Antibiotic cover (cephalexin Kenflex, 25 mg/kg/day) was given for at least 7 days or until there was no sign of urinary infection. Manual expression of the urinary bladder was performed three times per day until spinal reflex emptying was reestablished (about 1–2 weeks). After this stage, all operated animals were checked daily for the level of activity, food and water intake, skin condition, and locomotor function. Assessments of the reflexes were undertaken from 3 to 10 weeks, using the BBB scoring method (6). By this time spinal shock had passed and hyperreflexia was established.

RESULTS AND DISCUSSION

H Reflex in the Distal Muscles of the Foot under Ketamine Anesthesia

We first tested the effect of ketamine, a dissociative anesthetic which has been reported to have minimal inhibitory effects on spinal reflexes (22). For the initial anesthesia ketamine (120 mg/kg ip) was co-injected with xylazine (2 mg/kg) as a sedative. After the initial 20 min, additional ketamine injections were given at a dose of 25 mg/kg every 15 min to maintain a constant level of anesthesia as judged by the heart and respiration rates. Under this regime all withdrawal responses of forelimbs, corneal reflex, and whisker tremor were abolished. The heart and respiration rates were maintained at 352 ± 59 and 103 ± 16 ppm (mean ± SD), respectively.

Suprathreshold stimulation of the lateral plantar nerve gave rise to a short latency (M wave) response followed by a longer latency (H wave) response (Fig. 1A). In most preparations (7/10), the amplitude of the M wave was larger than the H wave at threshold (see Fig. 1B). Both the M and the H waves became more stable and increased in amplitude as the stimulation intensity increased. However, at higher stimulation intensity (>2 T), the amplitude of the H wave became suppressed and was always smaller than that of the M wave (Fig. 1B), presumably due to collision of antidromic and orthodromic efferent impulses (24). This stimulus-response relationship is a prominent feature of the H reflex. It was a concern that the H wave recorded in the present study did not always have a lower stimulation threshold than the M wave, as described in human studies (32). Although Meinck reported that in the foot muscles of the rat the H wave appeared to be the only response at threshold stimulation of the exposed tibial nerve (25), other studies of H reflex in the rat have reported that the H wave has a threshold similar to or slightly higher than that of the M wave (7, 14, 37).

Paired-Pulse Depression of H Reflex

The H reflex amplitude was also dependent upon the interpulse interval between control and test pulses (Fig. 1C). The peak-to-peak response of the H wave was reduced by more than 50% (arrow, Fig. 1C) when the interpulse interval was shortened to 100 ms. The paired-pulse depression of the H reflex was analyzed further by using a range of twin-pulse intervals from 5 s to 30 ms. The H response to the test (second) pulse was expressed as a percentage of that to the conditioning (first) pulse using the integrated area bounded by the respective H waves. We have divided the responses under different anesthetics into two groups according to the H-reflex rate-suppression profile.

Ketamine and halothane. The H-reflex response at different interpulse intervals under ketamine anesthesia is shown in Fig. 2A. As the interval is decreased from 5 to 1 s, the reflex shows a gradual reduction in amplitude to 25% of control values. The responses remained at 25% from 1 to 100 s, and then fell sharply. A similar response profile was found with light halothane (Fig. 2A). Unlike ketamine, halothane is known to block the H reflex in the human and the dog (24). We tested the effect of halothane on the H reflex in our rats and found that for animals maintained at a level of 1–1.5%, the H reflex was usually abolished. However, in some animals we observed intermittent responses with a latency similar to that of the H wave. This prompted us to reduce the anesthetic level, resulting in the H reflex being recorded satisfactorily within a narrow range of halothane concentration (0.5–1%). Spontaneous muscular movement remained absent at this stage but some animals showed a weak response to noxious paw pinch. The H response during twin-pulse stimulation showed a typical suppression profile, reducing to more than 50% when twin-pulse intervals were shortened to less than 1 s.

Ketamine has been the most commonly used anesthetic for studies on the H reflex and its fatigability (7, 33, 36, 37). Our present paired-pulse depression data have the same profile as those described by Thompson et al. (37) although with a lower level of depression (compare Fig. 6 of (37) to Fig. 2 here). This is likely due to the different experimental parameters in that our present study used twin-pulse stimulation with a rest
period of 20 s between successive trials while Thompson et al. used continuous stimulation at a particular frequency, giving rise to a more suppressed H response.

Etomidate, Saffan, and Nembutal. Figure 2B shows the results from animals anesthetized with Etomidate, Saffan, and Nembutal. Etomidate was developed in the 1970s by Janssen Pharmaceutica as a fast-acting hypnotic. Etomidate differs from most general anesthetics by acting mainly in the cerebral cortex while exerting minimal suppression on thalamic and brain-stem responses (2). Etomidate was very fast acting, producing a surgical level of anesthesia in our rats within 2–3 min after intraperitoneal injection at concentration of 20 mg/kg. Additional doses were given at half-strength (10 mg/kg) every 20–30 min to maintain suppression of withdrawal reflexes. The heart and respiration rates were in the range of 365 ± 47 and 68 ± 17 ppm, respectively. Myoclonus, an effect reported clinically, was not observed (n = 4), except in one animal during the recovery period. When interpulse intervals were progressively shortened to 100 ms, the response of the H wave remained above 75% (Fig. 2B, rectangles). Reduction of the H wave was observed when the interpulse interval was further shortened, reducing to about 25% at 30-ms interpulse interval.

A profile similar to that described above for Etomidate was also observed in four animals anesthetized with Saffan, a steroid anesthetic containing alpha-xalone and alpha-dolone acetate. The animals were first sedated using a dosage of 40 mg/kg followed by an additional injection 10 min later at the same dosage to deepen the anesthesia. Further injections were given after 20–30 min to maintain the anesthesia during the
Heart and respiration rates were in the range of 335 ± 52 and 69 ± 13 ppm, respectively. With twin-pulse stimulation, the test response remained unchanged until interpulse intervals were reduced below 100 ms (Fig. 2B, diamonds).

The result obtained from six animals anesthetized with Nembutal, a barbiturate, is shown plotted in Fig. 2B. The lower dose of Nembutal (30 mg/kg, ip) was necessary, rather than the normally used dose of 60 mg/kg, to prevent a blockade of the H reflex. The heart and respiration rates were 336 ± 48 and 76 ± 10 ppm, respectively. The H-reflex suppression curve showed an initial drop, as in the ketamine and halothane groups, followed by a transient increase, which peaked at 100 ms interpulse interval. Subsequent H responses were suppressed with shorter interpulse intervals.
Nembutal was the anesthetic chosen for investigations of H and flexor reflexes in a recent study by Duke and Advokat (10). They reported impaired rate-sensitive depression for both spinal and intact animals under Nembutal compared with unanesthetized animals. This would be entirely consistent with the findings here, with Nembutal conferring relative rate-resistance.

H-Reflex Rate Depression after Spinal Cord Injury

The H-reflex depression profiles observed in animals under Etomidate, Saffan, and Nembutal were similar to those previously reported for spinal-cord-injured animals (33, 37). We compared this directly by using our present recording procedure in spinal-cord-injured animals under ketamine anesthesia. Three animals were subjected to spinal cord impaction using the New York University weight drop impactor, with a drop of 50 mm. This resulted in an impaction velocity of 0.66–0.85 m/s and 1.9–2.1 mm of cord compression. All three animals showed hind-limb paralysis after the operation. The BBB scores (6) of the hind limbs remained below 3 and showed no signs of recovery during the 10-week study period. The H reflex was recorded from these animals from 3 to 10 weeks after the impaction operation.

Under ketamine anesthesia, a higher H/M ratio was observed in these three animals (11 recordings), consistent with the results of Duke and Advokat (10) for chronic spinal cord animals and with the development of hyperreflexia. At threshold stimulation, 7 of 11 experiments showed a larger H response (H/M > 1; data not shown). With increasing stimulus strength, the H response became smaller and the averaged H/M ratio was less than 1. The paired-pulse depression of the H reflex from these cord-impacted animals was less pronounced. The pooled result is shown in Fig. 2C. The colored trend lines in Fig. 2C are the same as in Figs. 2A and 2B and are repeated for comparison. After cord injury the H reflex showed a gradual decrease as the twin-pulse intervals were reduced. At the shortest twin-pulse interval of 30 ms, the spinal cord injury group had a paired-pulse depression of less than 50%. This was distinctly different from animals with intact spinal cords, irrespective of the anesthetic used. However, at longer twin-pulse intervals, the data indicate graphically the extent to which Saffan, Nembutal, and Etomidate mimic spinal cord injury. In contrast, halothane and ketamine stand out as producing a stronger paired-pulse depression profile in uninjured animals.

Effect of Ketamine Anesthetic Level and Respiratory Rate

Figure 2D shows the respiratory and heart rates recorded in these experiments under different anesthetics. While the heart rates were similar across different groups, the respiratory rates from animals anesthetized with ketamine and halothane were signifi-
cantly faster. Since these two anesthetics showed similar paired-pulse depression profiles (see Fig. 2A), one possibility was that the level of anesthetic, as reflected by the spontaneous respiration rate, may influence the H-reflex paired-pulse depression profile. We tested this idea by recording the depression profile under different levels of ketamine anesthesia (Fig. 3). The profile was similar under light ketamine anesthesia (series A, respiratory rate about 100–120 ppm, presence of withdrawal reflexes and rhythmic scratching in response to noxious pinch) and after an additional dose of ketamine (series B, respiratory rate 60 ppm, absence of any withdrawal reflex). We noted, like others (7), that H reflexes under light anesthesia became smaller and more variable and could be difficult to elicit. This is possibly due to an increase of inhibitory influences from interneuronal networks that underlie the locomotor central pattern generator, which imposes a presynaptic inhibition on the synaptic transmission between Ia afferents and motoneurons (13). Nevertheless, increasing the ketamine dosage level does not convert the H-reflex depression curve to a rate-resistant profile. These data suggest that the difference between paired-pulse depression profiles under the two groups of anesthetics is not simply due to the level of anesthesia.

Mechanisms of Anesthetic Action in Paired-Pulse Depression

The recovery of the H reflex following the conditioning stimulus will be affected by a range of mechanisms which include unloading of muscle spindles, inhibitory modulation via Renshaw cells, and Golgi tendon organ activation as well as presynaptic inhibition by primary afferent depolarization. These inhibitory mechanisms all have different time courses, contributing to the overall recovery seen here. For instance, GABAₐ-mediated presynaptic inhibition (34) has been implicated in rate-dependent depression for intervals of up to 200 ms (12, 19). There is evidence to support a role for GABAₐ-mediated presynaptic inhibition in longer lasting rate depression (35).

The recovery curves seen here under halothane and ketamine have time courses remarkably similar to those from normal unanesthetized humans following paired-pulse stimulation (28). With maximal stimulation, the recovery starts at interstimulus intervals of 20–30 ms, lasts for up to 5 s, and shows a similar plateau at approximately 200–500 ms. This suggests that halothane and ketamine might represent the recovery curves most closely corresponding in unanesthetized animals and would therefore be recommended as the anesthetics of choice in assessment of H reflexes.

Following spinal cord injury, the development of spinal hyperreflexia and rate-resistant H reflexes have been considered to relate to loss of presynaptic inhibition as a result of disruption of descending control paths (9). Thompson et al. (35–38) have argued for the modulation of GABAergic interneurons mediating presynaptic inhibition under these conditions. Such interneurons are known to be modulated by vestibulospinal, rubrospinal, and corticospinal paths, whose activation is known to have differential effects on primary afferent inhibition of Ia afferents (30, 31).

Olsen and Diamantopoulos (28) showed that spasticity in humans is associated with earlier recovery after paired-pulse stimulation. Conditioned responses following maximal stimulation reached 80% of test amplitudes with intervals of 200 ms or longer. This recovery is similar to that seen here under Saffan, Nembutal, and Etomidate and after spinal cord impaction. The suggestion is that these anesthetics affect the descending control paths, perhaps through an action on presynaptic inhibitory controls. Olsen and Diamantopoulos (28) also showed that disorders such as parkinsonism and cerebellar dystonia produced different H-reflex recovery patterns, suggesting selective modulation of descending paths by higher centers. Angel and co-workers have studied in detail the action of a range of anesthetics on sensory transmission and showed that most anesthetics, including the barbiturates, halothane, urethane, ketamine, and Saffan, depress transmission through the ventrobasal thalamus (1). In contrast Etomidate primarily affects cortical excitability (2). It is therefore unlikely that the H-reflex depression seen here is due to a common mode of action on sensory transmission. We suggest that these anesthetics have a differential mode of action on descending control paths and our present data suggest that modified GABAergic transmission may be critical for H-reflex rate resistance.

Barbiturates enhance the action of GABA at GABAₐ receptors (5) known to be involved in the rate-sensitive depression of spinal reflex by acting through a presynaptic mechanism (8). It is interesting to note that in the study by Duke and Advokat (10) the absolute amplitude of the H reflex in animals anesthetized with pentobarbital was smaller than that recorded in unanesthetized animals, suggesting an increased level of background inhibition as a result of the anesthetic. An enhanced GABAₐ background activity, as a result of the barbiturate anesthesia, may perturb further inhibitory action following repetitive afferent stimulation, resulting in a less pronounced increase in presynaptic inhibition and paired-pulse depression of the H reflex.

Etomidate enhances GABAergic activity by acting as a GABA mimetic and potentiates GABA-evoked currents (9, 17, 29). The steroid anesthetic Saffan also enhances the inhibitory effect of GABA through a positive allosteric modulation of GABAₐ receptors (4, 16). Saffan also produces an antinociceptive effect, but at a much lower dose and longer lasting time course than its anesthesia activity. Saffan’s analgesic action is me-
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...diated through a subpopulation of spinal GABA<sub>A</sub> receptors, which appears to be different from those that are involved in the neurosteroid-induced general anesthesia (26).

In contrast to the above results, the mechanisms of action for ketamine do not involve GABAergic modulation. Rather, ketamine has been shown to selectively act on NMDA-mediated responses while it has little effect on GABA and glycine-evoked activity (3). Halothane enhances GABAergic activity (27).

...injury by disrupting descending controls of GABAergic interneurons mediating PAD), and chemical injury (by disrupting descending controls of GABAergic presynaptic inhibition, such as spinal cord injury). Nishikawa et al. have reported that halothane enhances GABAergic activity (27).

Thus it is possible that procedures which modulate GABAergic presynaptic inhibition, such as spinal cord injury (by disrupting descending controls of GABAergic interneurons mediating PAD), and chemical interference with such transmission (e.g., barbiturate, Saffan, and Etomidate) both lead to a rate-resistant profile. In contrast, other anesthetics which do not involve GABA (e.g., ketamine) would appear to preserve paired-pulse depression relatively intact, although the complex mechanism of action of halothane, affecting both GABA and NMDA transmission, requires further investigation. Clearly the hypothesis of GABA's critical role in rate resistance of the H reflex will require validation with more direct assessment of interneuronal function at different stimulus intervals.

In summary, the present study highlights the variability of the monosynaptic spinal reflex under different anesthetics. As better regimens for limiting spinal cord injury and aiding regeneration become available, there is a pressing demand to develop techniques which can reliably assess cord function. Reflex excitability is being increasingly recognized as a valuable tool for assessing spinal cord injury and monitoring functional recovery (19, 33, 36). The current study highlights the feasibility of our recording procedure to obtain repeated H-reflex assessments from the same animal and the importance of anesthetic selection in studies of reflex excitability.

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