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Intrathecal Excitatory Amino Acid (EAA) Agonists Increase Tail Flick Latencies (TFLs) of Spinal Rats[1]

C. ADVOKAT,*[2] A. GHORPADE† AND E. WOLF*

*Department of Psychology, Louisiana State University, Baton Rouge, LA 70803
†Department of Psychiatry, Detroit Psychiatric Insitute, 1151 Taylor Ave., Detroit, MI 48202

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ADVOKAT, C., A. GHORPADE AND E. WOLF. *Intrathecal excitatory amino acid (EAA) agonists increase tail flick latencies (TFLs) of spinal rats.* PHARMACOL BIOCHEM BEHAV **48**(3) 693–698, 1994.—The facilitation of spinal nociceptive reflexes that occurs after spinal transection reveals the existence of descending, supraspinally mediated inhibition. Substantial evidence indicates that the excitatory amino acids (EAAs) are involved in these spinal circuits. Therefore, it was hypothesized that reflex facilitation in the spinal animal might be due to the removal of inhibitory input normally exerted on the spinal action of EAAs. If so, the facilitatory decrease in reflex latency, observed in the spinal preparation, might be potentiated by intrathecal (IT) administration of EAA agonists. This was tested by comparing the effect of IT injections of *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) on the thermally elicited tail flick (TF) response of Intact and acute spinal rats. In intact rats, a low (0.25 nM) dose of NMDA produced a hyperalgesic decrease in latency, relative to saline, whereas higher doses produced an overall increase in latency. A large dose (0.5 μM) produced overt signs of toxicity (crippling, self-mutilation, and loss of the reflex). Only the highest (1.0 nM) dose of AMPA affected the response, resulting in a significant increase. After spinal transection, the hyperalgesic reaction to 0.25 nM of NMDA was absent, and latencies were significantly increased by 1.0 nM. The toxic reaction to 0.5 μM appeared to be potentiated. Tail flick responses to AMPA were also significantly increased in spinal rats. Contrary to the prediction, reflex latencies were significantly increased by these drugs after spinal transection. It was suggested that, although the spinal action of EAAs appears to be supraspinally modulated, this influence may be facilitatory rather than inhibitory.

Excitatory amino acids Tail flick Spinal rats

FOR over 50 years it has been known that spinal reflexes are facilitated by spinal transection; that is, that both neural (11) and behavioral (15) responses are significantly potentiated in the spinal preparation. It is generally accepted that these effects of spinalization result from the loss of tonic inhibitory control, normally exerted by supraspinal input. At present, however, neither the origin(s) nor the neurochemical bases of tonic descending inhibition are well established.

On the other hand, recent studies have increased our understanding of the neuromodulation of spinal reflexes at the level of the spinal cord, particularly in regard to nociceptive processing. Within the last few years considerable evidence has accumulated implicating the excitatory amino acids (EAAs), particularly glutamate, in spinal nociceptive circuits. It is known that glutamate levels are high in the dorsal root ganglia and dorsal roots (10,28), that there is a high density of glutamatergic binding sites in the dorsal horn (13), and that glutamate is colocalized with substance P in primary afferents (7). Not only is glutamate released by peripheral application of various nociceptive treatments (14,16,26,27), but iontophoretic (1,6,9,24) and intrathecal (IT) (25) administration of EAA agonists increases the spontaneous activity and evoked responses of spinal neurons to peripheral noxious stimulation. Behavioral support for a role of EAAs in spinal nociceptive processing is indicated by the fact that IT injection of EAA agonists produces a syndrome indicative of pain in mice and rats (hindlimb biting and scratching within 1 min) (2,3). In addition, IT administration of the EAA agonist *N*-methyl-D-

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[2] To whom requests for reprints should be addressed.

aspartate (NMDA) produces a hyperalgesic effect both on the thermally elicited tail flick (TF) reflex of mice and rats (2, 17,20) and on the nociceptive response to SC formalin injection into the hindpaw (5). In contrast, these nociceptive reactions are not elicited by the non-NMDA excitatory amino acid agonist AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) (5,17).

With regard to possible modulation from supraspinal sites, there are several observations indicating that the spinal action of these drugs is influenced by descending input. First, it has been shown that the IT effects of NMDA on the thermally elicited tail flick withdrawal reflex are markedly altered by spinalization, in a manner that suggests it may be toxic (22) (i.e., that there may be a permanent loss of the reflex response in spinal rats given high doses of NMDA). Second, scratching behavior elicited by the endogenous EAA, L-glutamic acid, was potentiated after spinal transection in rats (4). Third, "pain" behavior elicited by IT NMDA was blocked by ICV morphine injection (8). Fourth, NMDA-induced neuronal excitation was reduced by noxious stimulation applied to an area remote from the receptive field of the neurons (25). This phenomenon, termed diffuse noxious inhibitory controls (DNIC), is supraspinal in origin. These reports suggest that the excitatory action of EAAs at the spinal level is suppressed by descending supraspinal inputs. One implication of these findings is that the facilitation of spinal reflexes produced by spinal transection may, in part, be due to the elimination of supraspinally mediated inhibition. If so, the effect of EAA agonists would be potentiated in the spinal preparation. The present studies were conducted to evaluate this hypothesis, by comparing the effect of IT-administered NMDA and AMPA on the thermally elicited tail-withdrawal reflex in intact and spinal rats.

METHOD

Subjects

A total of 133 male, albino Sprague-Dawley rats (Holtzman Laboratories, Madison, WI), weighing 300–450 g, were used as subjects. The animals were housed in suspended steel cages in a colony room maintained on a 12 L : 12 D cycle, with dark onset at 1900 h. Food and water were available ad lib.

Surgical Procedures

Intrathecal catheterization. Animals were anesthetized with a mixture of isoflurane (AErrane, Anaquest, Madison, WI) and oxygen and were placed in a stereotaxic frame. An incision was made behind the ears and the neck muscles were scraped to expose the back of the skull. An incision of the atlanto-occipital membrane allowed the insertion of an 8-cm long catheter of PE-10 polyethylene tubing filled with sterile saline into the spinal subarachnoid space. Prior to insertion, a loose knot was tied in the catheter and coated with dental cement so that it could be held in place against the skull with adhesive (Superglue; Bel-Art Products, Pequannock, NJ). The incision was closed and the exposed tip of the catheter was heat sealed. Any rat showing obvious neurological deficit (i.e., a crippled limb) postoperatively was eliminated from the study.

Spinal transection. In addition to the catheter implantation, several groups of rats (N = 62) also sustained a spinal transection. The skin incision was extended further and, after retraction of the paraspinal muscles, a laminectomy was performed between thoracic vertebrae 6 and 9. A 1–2-mm portion of the spinal cord was crushed and removed, leaving the catheter intact. The excised tissue was replaced with gel foam to reduce bleeding, after which the incision was closed in layers; the cages were placed on heating pads to maintain body temperature. On the morning after surgery, the hindquarters of each rat were washed with warm water and their urine was expressed manually by the application of pressure to their bladders. All experiments with spinal animals were completed within 24 h after surgery, whereas experiments on intact animals were completed within 1–7 days of surgery.

Behavioral Tests

Tail flick. The TF was used for nociceptive assessment (IITC Life Sciences, Woodland Hills, CA). Noxious stimulation was provided by a beam of high-intensity light focused on the tail. The response time was measured automatically and was defined as the interval between the onset of the thermal stimulus and the abrupt flick of the tail. Each determination consisted of three to five trials; the mean score was taken as the response latency. Animals not responding within the 14-s limit were removed from the apparatus to prevent tissue damage, and were assigned a score of 14 s.

Motor function. Subjects were placed on the mat of an inclined plane with the body axis perpendicular to the slope of the plane. The maximum inclination of the plane at which the rat could maintain itself for 5 s was recorded. Assessments were made in 5° increments; normal rats maintain their balance at an angle of 59 ± 3° (mean ± SD). An assessment was made on the inclined plane in studies using intact rats, prior to injections and between the 5- and 15-min test point. With the exception of the intact rats that received the highest (0.5 mM) dose of NMDA, only data from those rats with scores of 50° or better were included in the analyses.

Drug Administration

For IT injections, the tip of the catheter was cut, a 30-ga needle was inserted into the catheter, and 10 μl of the drug solution was infused followed by a 10-μl wash of the saline vehicle. Injections were performed manually with a 50-μl Hamilton syringe (Hamilton Co., Reno, NV) over a 2–3-min period.

On the day of the experiment, all rats were pretested on the tail flick apparatus for baseline assessment. Because spinalization produces a decrease in latency, it was appreciated that any additional facilitation (further decrease) produced by the drugs might not be detectable in spinal rats. For this reason, and also to be able to compare the data from intact and spinal rats directly, the beam intensity was adjusted so that the average latency for each experimental group (both intact and spinal) was 6.3–7.0 s for rats injected with NMDA, and 5.6–6.7 s for rats injected with AMPA. Each rat was then injected with either saline, NMDA (0.025–1.0 nM, or 0.5 μM) or AMPA (0.05–1.0 nM). Animals were then tested at 1, 3, 5, 10, and 15 min after injection.

Any rat with a score of 14 s at the 15-min test period was retested for recovery of the TF reflex to determine whether loss of the reflex was permanent. Each animal was used only once, and contributed a single pre- and postdrug data point.

Statistical Analyses

The effects of the drugs were assessed by Student's t-test and one-way and two-way analyses of variance (ANOVAs), performed with the aid of a computer program (CRUNCH

Interactive Statistical Program), followed by post hoc Newman-Keuls and Dunnett's tests. Analyses were performed on either: 1) TF latencies obtained at each time point, 2) the change in latency (difference scores) between baseline and each postinjection time point, or 3) the area under the curve (AUC). This value was obtained with the aid of a computer program (PHARM/PCS). For each animal the AUC was determined by entering each x and y data pair, in which x = TF latency and y = 1, 3, 5, 10, and 15 min. The computer program calculated the total area (i.e., the integral) based on an approximation using the trapezoidal rule. Statistical tests were then performed on the AUC values that comprised each experimental group. Results were considered significant at $p < 0.05$.

RESULTS

Figure 1 summarizes the effect of the highest (0.5 μM) dose of NMDA in intact and spinal rats. A repeated-measures ANOVA on these TF latencies indicated that there was a significant effect of condition [intact vs. spinal: $n = 20$, $F(1, 16) = 11.8$, $p = 0.0034$], drug [saline vs. NMDA: $F(1, 16) = 55.1$, $p = 0.0001$], and an interaction, $F(1, 16) = 11.0$, $p = 0.0043$. Because there was no difference betweeen the two saline-treated groups (intact and spinal), their data were combined for graphic presentation. These data show that, as reported previously (22), IT NMDA increases TF latency, and that this result is more pronounced in the acutely transected than in the intact rat. The significant interaction probably reflects the fact that within the first minute after injection, the scores of intact rats receiving NMDA showed a "hyperalgesic" decrease, whereas those of spinal rats immediately increased. In response to the saline injection, all (intact and spinal) rats showed a transient, slight, but consistent increase in response.

This dose of NMDA was clearly toxic. Two of the intact rats became crippled and could not perform the inclined plane test; one rat eventually died and two began to self-mutilate and never recovered the reflex. Of the two that did recover the reflex, one also began to self-mutilate. Of the five spinal rats, all showed muscular fasiculations and four of them did not recover the withdrawal reflex, although none died.

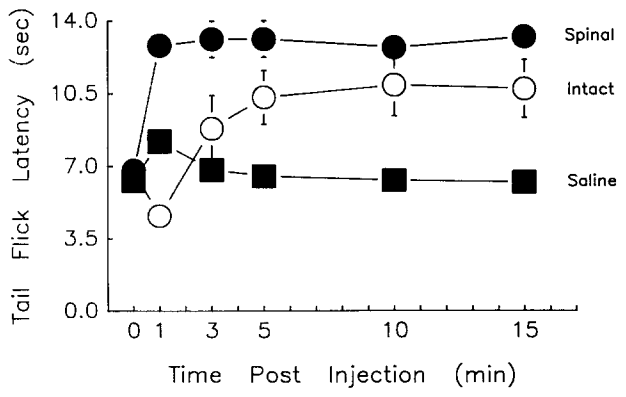

FIG. 1. The effect of IT-administered saline or 0.5 μM of NMDA on the tail flick of intact and acute spinal rats. Mean tail flick latency ± SEM of intact (open circles) and spinal rats (filled circles), prior to (time 0) and several time points after, an IT injection of either saline or NMDA. Because there was no difference in their response to saline, the data from the intact and spinal groups (filled squares) were combined for graphic presentation.

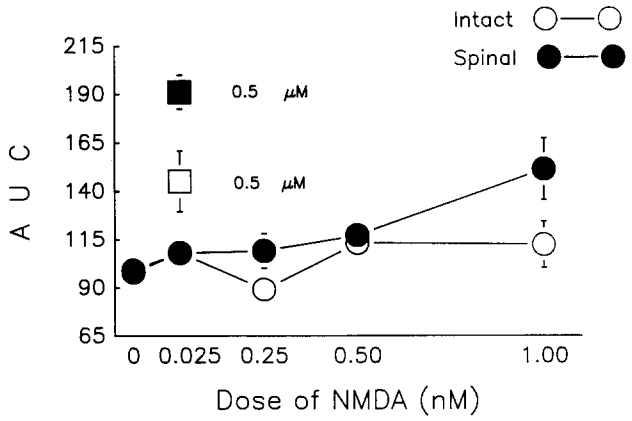

FIG. 2. The effect of different doses of IT-administered NMDA on the tail flick response of intact (open circles) and spinal rats (filled circles). Results are shown as the mean ± SEM AUC for separate groups of intact and spinal rats, injected with either saline or NMDA. The squares represent the corresponding AUC values of intact and spinal rats that received the highest dose of NMDA (shown in Fig. 1).

Results obtained with the lower doses of NMDA are shown in Fig. 2. In this study, the data from all time points were converted to a single AUC score for each rat. This figure summarizes the mean AUC scores for each of the five doses, in intact and spinal rats. In addition, the data shown in Fig. 1, for intact and spinal rats that received 0.5 μM of NMDA, were converted to AUC scores and included in this figure for comparison (open and filled squares, respectively). A two-way ANOVA, of the five lower doses, indicated a significant effect

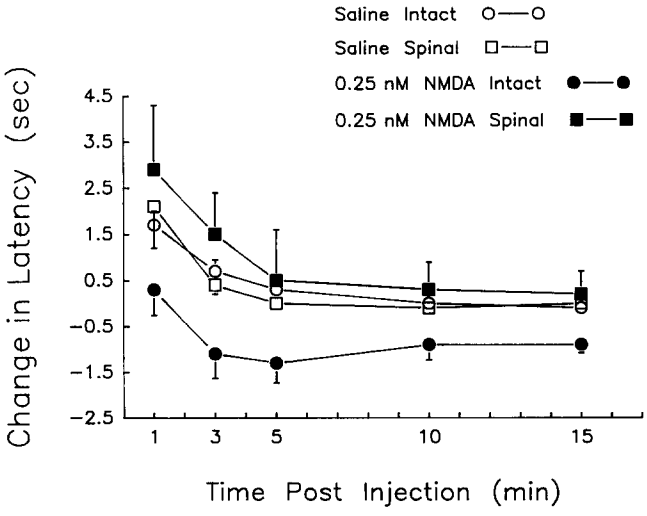

FIG. 3. The hyperalgesic effect of 0.25 nM of NMDA on the tail flick of spinal vs. intact rats. The data are summarized as the mean ± SEM change in latency from predrug baseline over the first 15 min after an IT injection of saline or NMDA. Statistical analyses indicated that NMDA elicited a hyperalgesic response in intact rats (filled circles) relative to saline (open circles). This effect was not seen in spinal rats (open vs. filled squares). Direct comparison of the two groups that received NMDA showed a significant difference between the intact and spinal rats (filled circles vs. filled squares).

of condition [intact vs. spinal: $n = 51$, $F(1, 41) = 7.3$, $p < 0.01$] and dose, $F(4, 41) = 5.95$, $p < 0.001$, with no interaction. This means that NMDA increased the latencies of spinal rats, relative to intact rats. A separate one-way ANOVA for intact rats showed a significant overall effect; however, no single group differed from any other. A one-way ANOVA on the scores of spinal rats also showed a significant effect of dose; in this case, the response of the group injected with 1.0 nM was greater than that of all other groups.

Inspection of Fig. 2 suggests that the 0.25-nM dose of NMDA produced a "hyperalgesic" decrease in latency in intact rats, which was absent in spinal rats. This is consistent with a previous report (2) that found this dose was hyperalgesic in intact rats, with respect to the effect of saline. Therefore, the data obtained with this dose in the present study was evaluated in the same manner as that reported by Aanonson and Wilcox. For each rat that received saline or 0.25 nM of NMDA, the difference in latency was determined between baseline and each postdrug time point. The change in latency, between the predrug score and the score at 1, 3, 5, 10, and 15 min after drug administration, was obtained for both the intact and spinal conditions. Repeated-measures ANOVAs were performed on these difference scores. These data are summarized in Fig. 3.

Analysis of the data from intact rats that received either saline (open circles) or NMDA (filled circles) showed that the decrease in latency produced by the drug was significantly greater than that produced by saline, $F(1, 8) = 11.8$, $p < 0.01$. A comparable analysis of the data for spinal rats (open and filled squares) indicated no difference, $F(1, 9) = 0.88$, NS. When the effect of this dose was directly compared between intact and spinal rats (filled circles and filled squares, respectively), the difference was also significant, $F(1, 9) = 7.8$, $p < 0.05$. These data are consistent with the results reported by Aanonson and Wilcox in intact rats, and additionally, they show that the hyperalgesia produced by a low dose of NMDA is eliminated after acute spinal transection.

The results of the IT injection of AMPA are summarized in Fig. 4. The data are again presented as the mean AUC scores for each of the doses in the intact and spinal groups. A two-way ANOVA indicated a significant effect of condition [intact vs. spinal: $n = 72$, $F(1, 60) = 6.4$, $p = 0.014$], dose, $F(5, 60) = 9.4$, $p < 0.0001$, and an interaction, $F(5, 60) = 3.0$, $p = 0.02$. A separate one-way ANOVA of the intact groups showed a significant effect of dose, $F(5, 35) = 5.7$, $p < 0.001$, with the 1.0-nM dose producing an increase that was greater than that of all others. The same analysis of spinal rats also showed a significant dose effect, $F(5, 25) = 5.9$, $p = 0.001$. In this case, the 0.25- and 1.0-nM doses produced a greater increase in latency than either saline, 0.05, or 0.125 nM.

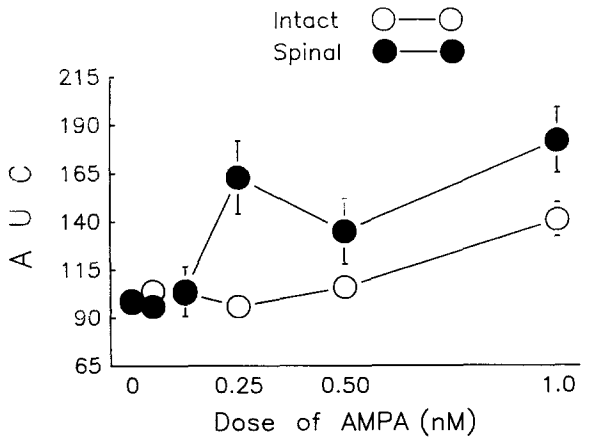

FIG. 4. The effect of different doses of IT-administered AMPA on the tail flick response of intact and spinal rats. Results are shown as the mean ± SEM AUC for separate groups of intact and spinal rats, injected with either saline or AMPA.

DISCUSSION

With regard to intact rats, the results of the present study are consistent with previous findings. Most of the available evidence concerning the spinal action of EAAs on nociceptive behaviors has been obtained with NMDA. In some cases [i.e., at higher doses (19,22,23) or longer time points (2,19)], spinal administration of NMDA produces an increase in the withdrawal latency of mice and rats to noxious thermal stimulation. This was also found in the present study, in that the one-way ANOVA for the AUC scores across all NMDA doses indicated a general increase (Figs. 1 and 2). However, at low doses (≤ 1.0 nM) NMDA produces a slight, but reliable, decrease in TF latency relative to saline injections (2,17,20). This was also seen in the present study (Fig. 3). As reported in mice (2), the 0.25-nM dose of NMDA produced a hyperalgesic reaction in intact rats. [In fact, as Aanonson and Wilcox also noted, saline produced an increase in latency within 1 min after injection (see Fig. 1).] Although it is not clear why the hyperalgesic effect was limited to a single dose, the general impression derived from the literature suggests that higher doses of NMDA may exert additional, nonnociceptive or nonsensory, effects on spinal reflex circuits. This is supported by the observation that the high 0.5-μM dose not only produced overt signs of aversion, but also significantly and permanently increased TF latencies, indicating a toxic effect on this behavior.

In contrast to NMDA, comparable doses of AMPA did not produce a hyperalgesic reaction in intact rats. This is consistent with other studies involving the TF reflex [(17); see (23) for a similar result with another agonist at this receptor, quisqualate], as well as formalin-elicited nociception (5). In contrast, Malmberg and Yaksh (19) observed a hyperalgesic effect 30 min after 1.1 nM of IT AMPA in rats, using the thermal paw withdrawal latency. This hyperalgesic reaction was preceded by an increase in latency, 5 min after injection. Although we examined the TF in several subjects for as long as 30 and 60 min after AMPA administration, we never observed a decrease in latency. On the contrary, in the present studies the highest (1.0 nM) dose increased latencies in intact rats relative to all of the lower doses.

As discussed in the Introduction, there are several pieces of evidence suggesting that the spinal action of EAAs may, under normal circumstances, be tonically suppressed by supraspinal input. The scratching and biting elicited by spinal application of L-glutamic acid is potentiated within 48 h after spinal transection (4). Nociceptive responses produced by IT NMDA are reduced by ICV morphine injection (8), and the neuronal excitation produced by IT NMDA on dorsal horn convergent neurons is reduced by noxious stimulation applied to areas of the body outside the receptive field of those neurons (25). These findings suggested that the effect of EAA agonists on the spinal cord might be potentiated in the absence of descending input.

To a certain extent, this is what happened. The action of both drugs was more pronounced in the spinal than in the intact rat. However, it was the increase in latency, rather than the (hyperalgesic) decrease, that was potentiated. In the case of NMDA, spinalization eliminated the hyperalgesic effect of the 0.25-nM dose, increased the effect of the 1.0-nM dose, and appeared to enhance the toxic effect of the 0.5-μM dose. This latter result is consistent with an earlier report (22), which also found that a high dose of NMDA increased the TF latency of intact rats, and that this was potentiated by spinalization. Spinalization produced a similar change in the effect of AMPA. In intact rats, only the highest dose tested (1.0 nM) increased reflex latencies. After spinalization, this drug was significantly more potent.

Although this outcome indicates that there is descending modulation of spinal EAA action, the nature of this supraspinal influence is difficult to characterize. There is compelling evidence that the EAAs are involved in spinal nociceptive processing. It is known that spinal nociceptive reflexes are facilitated after spinal transection. Therefore, it was hypothesized that the spinal action of EAAs might normally be suppressed by descending inhibitory systems, and that, when this input was removed, such facilitation would be potentiated by the exogenous administraton of EAA agonists.

The data do not support this hypothesis, and in fact, suggest that nociceptive reflex reactions are reduced, rather than increased, by EAAs in the spinal preparation. At least two types of explanations may be offered for these apparent anomalous results. On the one hand, the effect of EAAs in the spinal animal may reflect an action on efferent, as opposed or in addition to afferent, processes. In this regard, Bossut and colleagues (4) have suggested that scratching produced by spinal glutamate, and other compounds, might be "a manifestation of convulsive-like or spastic activation of a polysynaptic spinal reflex at a site efferent to the primary afferent synapse." Although there were no overt signs of scratching reactions to the low doses used in this study, it is possible that incipient efferent activity might have occurred, which interfered with performance of the withdrawal response.

On the other hand, the increased latencies produced by spinal EAAs in the spinal animal may, in fact, represent an increase in antinociception. This would support the proposed existence of descending facilitatory input (22). The possibility that spinal nociception may be modulated by descending "pain facilitatory mechanisms" (12,18) or antianalgesic systems (21) has recently received increased attention. Because EAAs are significantly involved in spinal pain networks, it is conceivable that they might provide a substrate for the mediation of descending facilitatory, hyperalgesic, or antianalgesic phenomena.

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