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Activation of Anti- and Pro-Nociceptive Mechanisms by Front Paw Shock in Spinal Mice: Involvement of Humoral Factors

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MAREK, P , I PANOCKA AND B. SADOWSKI *Activation of anti- and pro-nociceptive mechanisms by front paw shock in spinal mice Involvement of humoral factors* PHARMACOL BIOCHEM BEHAV 24(4) 791–793, 1986 —The effect of prolonged, intermittent front paw shock on nociception was studied in two groups of differently spinalized mice The animals, spinalized so that the dura was left intact to allow free cerebrospinal fluid (CSF) passage, exhibited post-foot shock increase in latencies of spinally-mediated nociceptive reflexes This anti-nociception was completely blocked by naloxone A facilitation of nociceptive reflexes was observed in animals in which the spinal cord was ligated together with the dura. The results indicate that (1) front paw shock in mice leads to activation of supraspinal sites which mediate anti-nociception by releasing substance(s) reaching the spinal cord via the CSF route, (2) single stressors may simultaneously activate both anti- and pro-nociceptive mechanisms

Footshock Anti-nociception Hyper-nociception Mice Humoral mediation

It is well established that noxious or stressful situations cause an increase of pain threshold in rodents [5, 6, 7, 8, 19] This phenomenon does not rely on a single mechanism, but different experimental stressors may activate separate pain-inhibiting pathways [19] Also, the single stressor, inescapable footshock, was found to produce different forms of analgesia when delivered in different paradigms For example, analgesia caused by long-lasting footshock was blocked by naloxone and showed cross-tolerance with morphine which indicates endogenous opioid mediation [6,7]. In contrast, analgesia produced by exposure to short-lasting footshock was found to be non-opiate in nature [6,7]. The form of the footshock induced analgesia is also dependent upon the body region shocked As demonstrated by Watkins *et al* , front paw shock produced analgesia which is naloxone reversible and shows cross-tolerance with morphine, whereas hind paw shock produces non-opiate analgesia [17,19].

In spite of these complexities, all of these forms of footshock-induced analgesia exhibit some common patterns Thus, lesion studies showed that both opiate and non-opiate analgesia are at least partially dependent on the integrity of the spinal dorsolateral funiculus [8] Destruction of the descending pathway also abolishes or attenuates analgesia produced by electrical brain stimulation, morphine and classically conditioned analgesia [1, 5, 17] These findings strongly support the concept that transmission of nociceptive information is controlled by supraspinal structures at the level of the dorsal horns [19]

However, the surprising results, with regard to the role of the descending pathways of the spinal cord, were obtained by Fu and Dewey in their study on morphine anti-nociception in mice Ligation of the spinal cord, but not spinalization with free spinal CSF flow maintained, totally abolished the morphine nociceptive threshold elevation [3]. It was suggested that morphine anti-nociception in mice, unlike in the rat, is mediated by endogenous substances which are released at the supraspinal level and reach the spinal cord via the CSF

The present study was undertaken to determine whether a similar mechanism might be involved in analgesia induced by stressful stimuli

METHOD

Surgery and Experimental Procedures

The experiments were performed on two groups of Swiss strain mice (average weight=30 g) spinalized differently according to the method published by Fu and Dewey [3]. The surgery was performed under pentobarbital anaesthesia. In one group of animals (DURA-INTACT), after laminectomy of T 11, 12 and 13 vertebrae, the spinal cord was elevated by a small hook and then squeezed through the dura so that its continuity was completely broken The dura was left intact to allow free flow of the CSF to the lower parts of the spinal cord In the second group of animals (LIGATED), the course of surgery was similar, but instead of squeezing, the

spinal cord was ligated together with the dura with silk thread This preparation blocked the flow of the CSF to the caudal portions of the spinal cord

Two weeks after the surgery, 31 DURA-INTACT and 27 LIGATED animals were exposed to 30 min of inescapable footshock A 1 mA current was delivered to the grid floor on 10 sec ON/10 sec OFF schedule During the stress, the hind part of the mouse body was elevated so that only front paws were stimulated The electric circuit was closed through an electrode inserted under the skin in the neck area

One hour prior to footshocking, 14 animals in each group received physiological saline whereas the others were preinjected with naloxone (1 mg naloxone-HCl [Endo]/0 3 ml 0 9% saline/kg body weight, IP)

The nociceptive threshold was determined as the mean response latency to radiant heat focused on a spot 20 mm from the tip of the tail. The first movement noted, i e , either withdrawal of the tail or characteristic tail base wriggling which usually appeared simultaneously with hind paw wriggling was regarded as a response

The measurements were taken immediately prior to (baseline) and then in the 1st, 5th, 10th, 20th and 30th minutes post-shock The average latency for each indicated time point was calculated as a mean of four measurements separated by 30-second intervals The light intensity was adjusted to keep the average baseline response at about 2 sec in all animals (2 21 sec, ±S E M =0 06, 84 animals) A 5 sec cut-off time was applied to avoid tissue damage The measurements were performed as a blind procedure, i e , the experimenter was unaware of either the surgery or the drug manipulation being tested

Twelve DURA-INTACT and 14 LIGATED mice were exposed to nociceptive threshold testing in an identical temporal paradigm after being preinjected with saline but not shocked

In five stressed DURA-INTACT mice, in spite of applying the cut-off time, tail skin blistering was observed In these cases the preblistering values only were included in the calculations.

Completeness of spinalization was confirmed histologically in several mice from both groups two weeks after surgery. A fragment of the spinal cord was excised, placed in 10% formaline and embedded in celloidin Oblique sections were stained after the Weil technique [12] No myelinated fibers were seen in the area of transection

Statistics

Changes in average nociceptive latencies relative to baseline were subjected to three-way analysis of variance The two groups of differently spinalized animals (spinalization factor) and naloxone + footshock (procedures factor) were taken as independent, and the changes in nociceptive threshold (test factor) as repeated measures Individual comparisons were based on F ratios for simple effects

RESULTS

Overall analysis of variance showed significant differences between differently spinalized animal groups, $F(1,78)=16\ 48$, $p<0\ 001$, between procedures, $F(2,78)=5\ 61$, $p<0\ 01$ and between tests, $F(4,286)=4.25$, $p<0\ 001$, and a significant spinalizations × procedures × test interaction, $F(10,286)=5\ 63$, $p<0\ 001$ Detailed results are presented in Fig 1 A significant increase in nociceptive threshold was seen in the DURA-INTACT group 5–30 min, but not immediately after footshocking This anti-

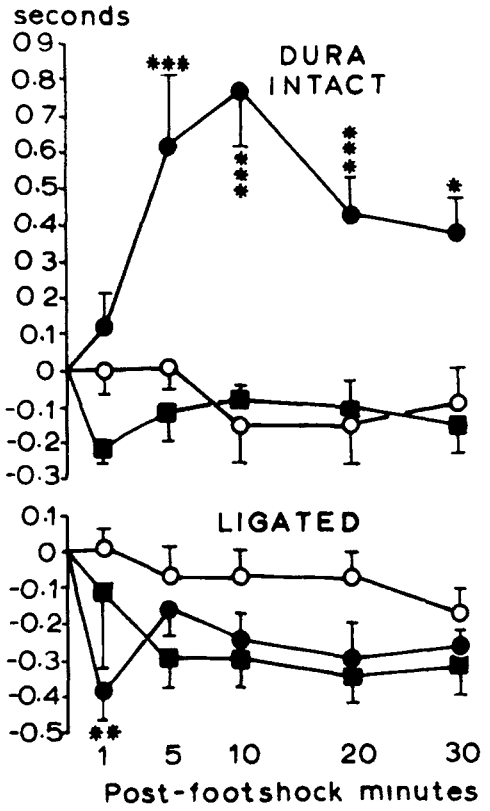

FIG 1 Mean changes (±S E M) in nociceptive threshold latencies after 30 min of front paw shock in mice spinalized in the way to leave or to block the passage of CSF to caudal portion of the spinal cord NaCl/footshock (filled circles) and naloxone/footshock (squares)—mice were injected before the shocks with physiological saline or 1 mg/kg naloxone-HCl NaCl/no footshock (open circles)—mice were not shocked but were preinjected with saline and tested for nociceptive threshold at the same time schedule *$p<0\ 05$, **$p<0\ 01$, ***$p<0\ 001$ versus NaCl/no footshock (based on F ratios for simple effects)

nociceptive effect was completely reversed by naloxone Post-naloxone/footshock nociceptive latencies did not differ from those following NaCl/no footshock and were significantly shorter than post-NaCl/footshock values In the LIGATED group, footshock produced a transient decrease in nociceptive threshold seen at the first measurement only Naloxone partially reversed this effect, $F(1,370)=0\ 47$ and 3 69 vs NaCl/no footshock and NaCl/footshock, respectively, $p>0\ 05$

DISCUSSION

Two different effects of front paw shock on nociceptive threshold were found in spinal mice depending on how spinalization was performed (1) when neural pathway from supraspinal centers to the caudal portions of the spinal cord were damaged, but the CSF flow was maintained, footshock produced an inhibition of nociceptive responses, (2) when both neural and humoral connections were interrupted, facilitation of nociceptive responses was seen after footshock.

Although the magnitude of footshock-produced antinociception in DURA-INTACT mice was quite small, it was similar to which we had observed earlier in experiment carried out on intact animals (unpublished data)

Since footshock-produced anti-nociception disappears after interruption of both CSF and neural connections to the spinal cord and persists when the CSF route is intact, it seems likely that the anti-nociceptive effect of footshock is mediated, at least partially, by CSF factor(s) released at the supraspinal level that descend along the subarachnoid space to the dorsal horns This mechanism is similar to that proposed by Fu and Dewey for morphine analgesia in mice [3].

The chemistry of this anti-nociceptive factor(s) is still to be determined A complete reversal of the front paw shock-produced anti-nociception by naloxone indicates a possible role of endogenous opioids This assumption is justified in the light of reports of the presence of endogenous opioids in the CSF [2,14]. Moreover, opioid compounds were found to act at the spinal level [15]

Because of the inadequacy of the available data, it is only possible to speculate which supraspinal structures are the source of the substances(s) that produce the anti-nociceptive effect of stress. The putative candidate is the pituitary since it was found to be the source of β-endorphin released in response to acute stress [4] Our previous results demonstrating the enhancement of stress-induced analgesia in adrenalectomized mice also indirectly implicate pituitary substances as possible mediators of this effect [9,10]

The decrease of post-footshock latencies in mice with a ligated spinal cord suggest that: (1) the stressing procedure used in this study activates not only an anti-nociceptive mechanism but also a reversed one, (2) facilitation of nociceptive reflexes is exerted at the spinal level by factors reaching spinal sites through the blood stream. The simultaneous activation by stress of anti-nociceptive and pro-nociceptive mechanism has been suggested by Vidal *et al* [16] who found stress-induced hyper-algesic response in hypophysectomized rats. They hypothesized that this response was due to the elimination of pituitary analgesic substances which compensated for a hyper-nociceptive response in intact animals. The present results support this hypothesis of dual action of a single stressor on nociception. They further indicate that hyper-nociceptive mechanisms may be activated not only by mild non-noxious stressors but also by more severe ones.

It is important to emphasize that there is no alteration of nociceptive threshold in the DURA-INTACT group immediately after footshock, i e., at the time when the LIGATED group exhibited a decrease in nociceptive threshold latencies We assume that this may be due to the compensatory action of a pro-nociceptive mechanism, operating also in the DURA-INTACT group, but masked by the prevailing anti-nociceptive effect of stress.

The question arises as to what mechanism(s) mediates the stress-induced hyper-nociception Its attenuation by naloxone might suggest involvement of an endogenous opioid system. Interestingly, a similar naloxone effect was found in the case of hyper-algesias induced by tail-pinch and prolonged intermittent footshock [11,13].

In conclusion, the present results provide additional evidence for a physiologic multiplicity of nociception-modulating mechanisms. They also support our previous suggestions that these mechanisms may differ between rats and mice [10]

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