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Immobilization-Induced Analgesia: Possible Involvement of a Non-Opioid Circulating Substance

H. A. JØRGENSEN,[1] O. B. FASMER, O.-G. BERGE
L. TVEITEN AND K. HOLE

Department of Physiology, University of Bergen, Bergen, Norway

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JØRGENSEN, H. A., O. B. FASMER, O.-G. BERGE, L. TVEITEN AND K. HOLE. *Immobilization-induced analgesia: Possible involvement of a non-opioid circulating substance.* PHARMACOL BIOCHEM BEHAV **20**(2) 289–292, 1984.— Stress induced analgesia (SIA) and stress-induced changes in body temperature were studied in mice and rats. Immobilization was used as the stressor. Nociception was measured with the tail-flick method and body temperature was recorded in the colon. Within 5 min of immobilization a similar increase in tail-flick latencies was observed in the two species. Concomitantly, the body temperature increased in the rats and decreased in the mice. Transection of the spinal cord 7 days before the experiments tended to increase the effect of stress on the tail-flick latencies in both species. Pretreatment with naloxone HCL (2 mg/kg SC, 5 min before immobilization) did not influence SIA in either intact or spinal rats. Thus, analgesia induced by immobilization may be due to a non-opioid substance acting peripherally or reaching the spinal cord via the systemic circulation.

Stress Immobilization Analgesia Body temperature Naloxone Spinal rats Spinal mice

SEVERAL studies have shown that elevated pain thresholds may be induced in rodents by subjecting them to stressful situations. This phenomenon, called stress-induced analgesia (SIA), seems to be produced by opioid [1, 2, 8, 10, 17, 18, 19] and non-opioid [6, 7, 13, 21, 22, 26] mediators acting via hormonal or neural pathways. The nature of the mediator depends upon the choice of stressful procedure and in some cases also upon the length of time the stressor is imposed on the animals [12,16]. Immobilization-induced analgesia in rats has been proposed to be mediated by a pituitary, opioid hormone [2,3]; but also non-opioid substances have been suggested [5]. It has not been shown whether the mediators inhibit nociception by activating supraspinal sites or whether they act directly at the spinal level. It is also possible that changes in function of neuronal membranes, due to stress-induced changes in body temperature [14, 15, 23], may influence nociception.

In the present study immobilization-induced analgesia was elicited in mice and rats. Both normal and spinally transected animals were used and the tail-flick test was employed to measure the spinal reflex sensitivity to noxious stimulation. By investigating SIA in spinal and intact animals, with and without the use of an opiate receptor antagonist, it should be possible to gain information concerning opioid systems as mediators of immobilization-induced analgesia, and the route by which the mediators reach the target area. In addition, body temperature was measured in the two species in order to compare changes in temperature with changes in nociception.

METHOD

Animals

Male Sprague-Dawley rats (Møllegaard, Denmark), weighing 200–300 g, and male NMRI mice (University of Bergen, Norway), weighing 30–35 g, were used. The rats were housed individually: the mice were kept in colony cages, 6–10 in each cage. The animals had free access to food and water. All experiments took place during the light-phase which lasted from 8:00 to 20:00 hours. Ambient temperature was 23°C.

Surgery and Drugs

Rats assigned to the spinal groups received anaesthesia of pentobarbital (40 mg/kg) and chloral hydrate (180 mg/kg) IP. The transection was performed as follows: the level of Th_{9-10} was ensured by palpation and proc. spinosi and laminae were exposed through an incision. One lamina corresponding to Th_9 or Th_{10} was removed with a dental burr and the spinal cord was cut with fine scissors. The blood was carefully removed by suction before closure with sutures. In the mice, the spinal cord was transected at the level of Th_{7-8} under anaesthesia of pentobarbital (35 mg/kg) and chloral hydrate (150 mg/kg) IP, according to the procedure described above. Behavioural experiments took place 7 days after surgery. Rats treated with naloxone received naloxone HCl 2 mg/kg SC. The drug was dissolved in saline (2 mg/ml).

[1]Requests for reprints should be addressed to Hugo A. Jørgensen, MD, Institute of Physiology, Årstadveien 19, N-5000 Bergen, Norway.

Test Procedure and Stress Induction

The spinal reflex sensitivity to noxious stimulation was measured with the tail-flick test using an IITC INC. Mod. 33 Analgesia-meter. Radiant heat was focused on a spot 1–2 cm from the tip of the tail. Beam intensity was adjusted to give a reaction time of 3–4 sec in intact control animals. In agreement with previous observations, spinalization shortened the tail-flick latencies in controls by approximately 25% [4].

Body temperature was measured in the colon by means of a thermistor probe inserted 3 cm (mice) or 5 cm (rats) into the rectum.

Stress was induced by immobilization. The animals were taped in a supine position (tail free) to rectangular pieces of perspex (9×30 cm). This arrangement made it possible to test the animals while immobilized. The controls remained in their cages until testing.

Protocol

In the first experiment 14 rats were used in a cross-over design. All animals received anaesthesia and 6 were spinally transected. During the following 7 days all animals were daily handled and trained in the tail-flick test situation. After the recovery period the animals were divided into a stress group and a control group with 4 intact and 3 spinal rats in each group. The basal tail-flick latencies were obtained in both groups 5 min prior to the session which was initiated by immobilization of the stress group. The testing was again performed on both groups 5 min into the session and repeated at 15 min intervals. The immobilization period was 35 min and the entire session lasted 80 min. The following day the conditions for the two groups were reversed and the procedure repeated.

In the second experiment 20 mice were used in a cross-over design. A laminectomy was performed on all the animals. In addition, the spinal cord was transected in half the number of mice. After recovery, the animals were divided into two groups with 5 intact and 5 spinal mice in each group. The same experimental procedure was followed as in the first experiment except for some changes in test intervals and observation time as shown in Fig. 2.

In the third experiment, body temperature was obtained in intact rats and mice during stress and control conditions. There were 10 animals of both species in each group. The observation period was 150 min and the test intervals were 30 min. Basal values were obtained 30 min before start of the experiment.

In the fourth experiment 30 rats were used in a cross-over design. All animals were anaesthetized and 12 rats were spinalized. After recovery and training in the tail-flick test, the animals were divided into a stress group and a control group. Each group consisted of 9 intact and 6 spinal animals. In this experiment the stress group was subdivided so that half of the intact and half of the spinal rats received naloxone 2 mg/kg SC, 5 min before initiation of the observation period, the remainder received saline only. The observation period was 50 min. The next day the conditions for the stress and non-stress groups were reversed. Other experimental procedures were as described above.

The testing was performed without knowledge of the drug treatment.

Statistics

Basal values were evaluated with one-factor ANOVA.

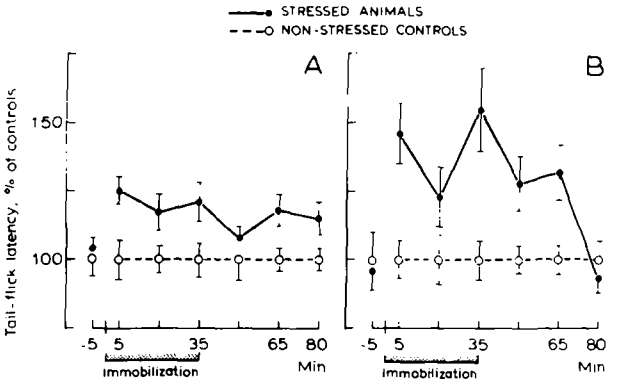

FIG. 1. Tail-flick latencies of rats exposed to immobilization stress. Data are expressed as % of the latencies of the control group, which was tested without being immobilized. Mean±S.E. A: Intact rats ($p<0.01$, stress vs. control group, n=8/group); B: Spinal rats ($p<0.05$, stress vs. control group, n=6/group).

The experimental values were analyzed by means of a two-factor ANOVA with repeated measurements on one factor. The limit of confidence was set at the 5% level. In the figures, data from the stress groups are expressed as percent of their corresponding controls. The statistical evaluation however, was performed before this transformation.

RESULTS

The first experiment showed that immobilization of intact rats produced an elevation of tail-flick latencies, approximately 25% above the level of non-stressed controls (Fig. 1A). The increase was evident 5 min after the animals had been immobilized, and had not returned to control level at the end of the observation period, $F(1,14)=14.46$, $p<0.01$, stress versus control group, basal values were not significantly different. The same procedure caused an even more pronounced increase in tail-flick latencies of spinal rats, the maximum was approximately 50% above control level, $F(1,10)=8.10$, $p<0.05$, stress versus control group, basal values were not significantly different. This increase subsided approximately 45 min after termination of immobilization (Fig. 1B).

In the second experiment immobilization of intact and spinal mice confirmed the results obtained in rats (Fig. 2). The intact stressed mice showed approximately 30% increase in tail-flick latencies compared to their controls $F(1,18)=19.06$, $p<0.001$, stress versus control group, basal values were not significantly different. The stressed spinal mice showed an increase in tail-flick latencies reaching a maximum of approximately 50% compared to their controls $F(1,18)=60.34$, $p<0.001$, stress versus control group, basal values were not significantly different. This was in agreement with the results obtained in spinal rats. The increase in tail-flick latencies of the stressed mice started to subside during the last part of the immobilization period and the latencies were normalized after termination of the stress.

The immobilization induced an increase in body temperature in rats (Fig. 3A), whereas a decrease was measured in mice (Fig. 3B). The difference between the control and the

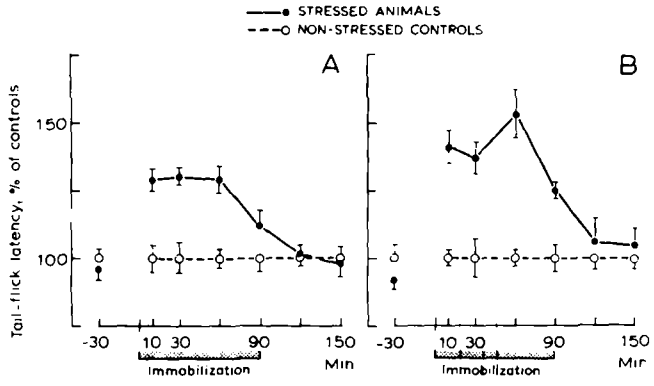

FIG. 2. Tail-flick latencies of mice exposed to immobilization stress. Data are expressed as % of the latencies of the control group, which was tested without being immobilized. Mean±S.E. A: Intact mice: B: Spinal mice ($p<0.001$ stress vs. control groups, n=10/group).

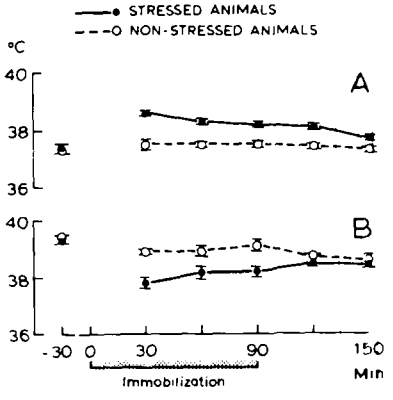

FIG. 3. Body temperature in intact rats (A) and mice (B) exposed to immobilization stress and in non-stressed controls. Mean±S.E. (rats: $p<0.001$, mice: $p<0.01$; stress vs. control groups, n=10/group). Ambient temperature was 23°C.

stress group was statistically significant in both species (rats, $F(1,18)=46.31$, $p<0.001$; mice, $F(1,18)=10.02$, $p<0.01$, stress versus control groups. Basal values were not significantly different).

Figure 4A shows that the stress-induced increase in tail-flick latencies in intact rats was not significantly reduced by naloxone. Similarly, naloxone did not reduce the increased nociceptive thresholds in stressed spinal rats (Fig. 4B). Basal values were not significantly different.

DISCUSSION

Immobilization in a supine position induced a moderate increase in nociceptive thresholds as measured with the tail-flick method, both in rats and mice. During immobilization, body temperature increased in the rats and decreased in the mice.

The increase in nociceptive threshold was observed within 5 min of immobilization and the tail-flick latencies remained prolonged at least as long as the animals were immobilized. This is in agreement with previous studies in rats

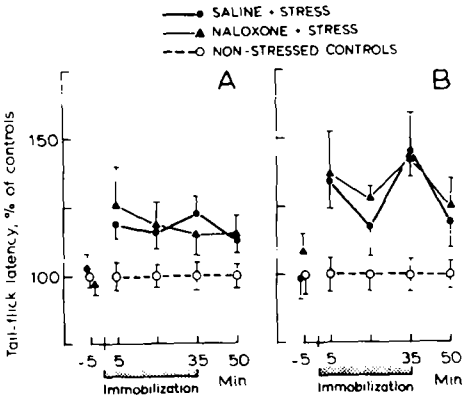

FIG. 4. Tail-flick latencies of rats injected with naloxone 2 mg/kg SC or saline 5 min before immobilization stress. Data are expressed as % of the latencies of non-stressed control groups. Mean±S.E. A: Intact rats (n=9 in both the naloxone and the saline group, n=18 in the control group); B: Spinal rats (n=6 in both the naloxone and the saline group, n=12 in the control group). Naloxone and saline groups did not differ significantly.

[2, 3, 5]. Immobilized spinal animals of both species not only showed prolonged tail-flick latencies, but the effect tended to be more pronounced than that observed in the immobilized intact animals (50–55% versus 25–30%). However, exact comparison of spinal and intact animals is difficult due to different basal values. The results in spinal animals are in contrast to previous studies which found that spinal rats displayed reduced SIA elicited by electric shock, by centrifugal rotation and by classically conditioned fear [7, 13, 26]. The cited studies also employed the tail-flick test. In preliminary studies we found that minor changes in experimental conditions may influence SIA in the NMRI mice and that C57Bl/6J mice are less sensitive to such changes. Thus, differences in type of stressor, experimental design and strain of animals may underlie differences in results.

Several studies on the neurochemical correlates of SIA have focused on the distinction between opioid and non-opioid mechanisms [6, 7, 16, 26]. Recent data [20] suggest that the analgetic effect of all opiate alkaloids and opioid peptides is mediated by a single high-affinity and naloxone sensitive binding site (mu_1). Another recent study [25] concludes that spinally mediated analgesia (tail-flick test) involve both mu and delta receptors and that naloxone 0.4 mg/kg IP prevents the analgetic effect of the delta agonist D-ala^2, D-leu^5-enkephalin given intrathecally. In our paradigm we had no effect of naloxone (2 mg/kg) either in intact or spinal rats. This argues against involvement of an opioid mechanism, although we cannot exclude that SIA is mediated by an opioid substance acting on a receptor which has low affinity for naloxone.

Several studies [11, 14, 24] have related the CNS effects of anaesthetics, alcohols and barbiturates to their ability of increasing the cell membrane fluidity. A rise in temperature will also increase the fluidity of membrane lipids [14, 15, 23]. Increased tail-flick latencies might in this way be caused by increased body temperature. Our results show a similar increase in tail-flick latencies in rats and mice, whereas the body temperature is affected in opposite direction in the two

species. Therefore, SIA does not seem to be dependent on increased body temperature.

In conclusion, data in the present study are consistent in two species. The results exclude pathways between the brain and the spinal cord as necessary for immobilization-induced analgesia measured by the tail-flick method. The study also indicates that stress-induced increase in body temperature, resulting in changes in membrane fluidity, is not a prerequisite for SIA. The best interpretation of the data seems to be that the mediator of the observed analgesia is a non-opioid mechanism located peripherally or in the lower spinal cord, possibly a substance that reaches the receptor area via the systemic circulation. The exact nature of the mechanism remains to be established.

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