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Enhancement of Stress-Induced Analgesia in Adrenalectomized Mice: Its Reversal by Dexamethasone

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MAREK, P , I PANOCKA AND G HARTMANN *Enhancement of stress-induced analgesia in adrenalectomized mice Its reversal by dexamethasone* PHARMAC BIOCHEM BEHAV **16**(3) 403–405, 1982 —Adrenalectomy significantly increased the level of analgesia induced by room temperature swimming in mice, as revealed by a hot-plate test This augmentation of antinociceptive action of stress was abolished by dexamethasone pretreatment Involvement of pituitary opioids in modulating post-stress pain sensitivity in mice is suggested

Stress Analgesia Pain Adrenal cortex Dexamethasone

ACUTE exposure to stressful stimuli increases pain threshold in the rat and mouse Electric foot shock [1, 8, 14, 15, 19], cold water swimming (2, 8, 15°C/3 5 min) [2, 4, 5], centrifugal rotation [14,15], conditioned fear [7] and room temperature water swimming (20°C/3 min) in mice [28] produce pronounced analgesia All of these procedures stimulate pituitary-adrenal activity [20,21] Several studies indicate pituitary mediation of pain responsiveness in stressed animals Disrupting the pituitary-adrenal axis by hypophysectomy was found to attenuate analgesia induced by immobilization [2], insulin [6], cold water swimming stress [5], acupuncture [23] and prolonged foot shock [11,22] In addition, analgesia induced by cold water swimming stress, foot shock [12] and front paw shocks was shown to be potentiated by adrenalectomy Moreover, analgesia induced by prolonged foot shock was blocked by dexamethasone in intact rats [18]

In the present study we report that the enhancement of swim-induced analgesia in mice produced by adrenalectomy is counteracted by dexamethasone pretreatment

METHOD

Male CFW-strain mice weighing 30 g in average, reared at natural day-light cycle were used In 16 mice a bilateral adrenalectomy and in 17 others a sham operation (which consisted of incising and suturing the skin without removing the glands) were performed under ether anesthesia Mice were kept five to a cage at 25°C and given physiological saline (0 9% NaCl solution) and food ad lib Completeness of the adrenalectomy was assessed through autopsy at the end of experiment

Swimming was used as a stressful stimulus Each mouse swam in 20 cm deep water at 21°C for 3 min and then was placed for 2 min in a box lined with gauze to dry off Pain sensitivity was assayed on a hot plate at 56°C just before swimming (pre-swim) and immediately after 2 min drying (post-swim) [28] The criterion was a hind paw flick whose latency was measured with a stop watch Animals which failed to respond within 60 sec (the cut-off time) were removed from the hot plate

Pre- and post-stress nociceptive latencies were measured a day before the surgery and on the 8th and 9th post-operative days (post-op 8 and post-op 9, respectively) On the 8th day all animals were injected with physiological saline (0 3 ml 0 9% saline, IP) On the 9th day 11 adrenalectomized (ADREX-DEXA group) and 12 sham operated (SHAM-DEXA) mice received dexamethasone (0 5 mg Decadron Phosphate [Merck Sharp and Dohme]/0 3 ml 0 9% saline/kg body weight, IP), whereas all other (ADREX-NaCl and SHAM-NaCl) groups were treated again with saline All injections were made 100 min before pain threshold determinations A comparable dexamethasone protocol is known to reverse opiate hypersensitivity in adrenalectomized mice [16]

Three-way split-plot analysis of variance with cross-

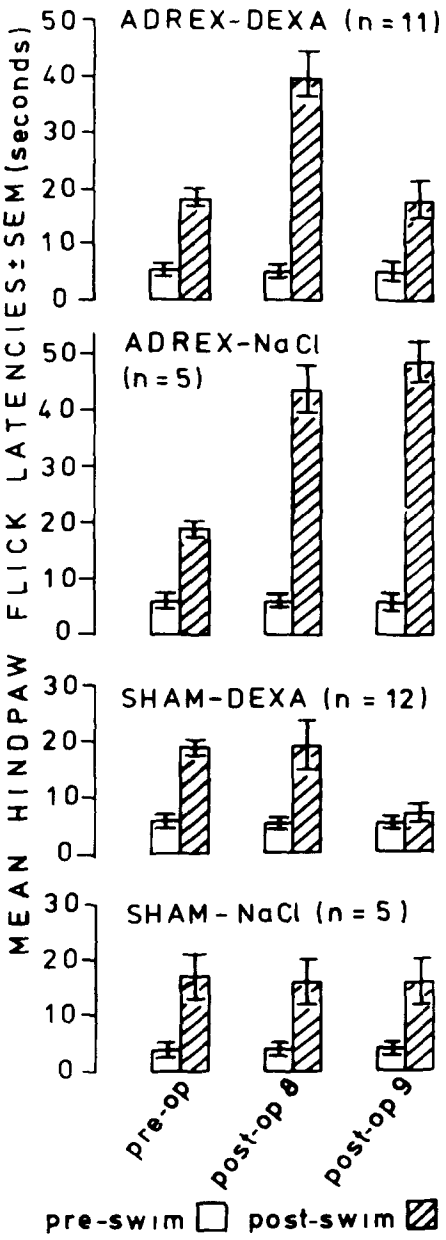

FIG 1 Pain thresholds before and after swim stress pre-op—before the surgery, post-op 8—on the 8th and post-op 9—on the 9th day following the removal of the adrenals (ADREX) or after a sham adrenalectomy (SHAM) Each group is labelled according to the surgery and preinjection with dexamethasone (-DEXA) or 0 9% NaCl (-NaCl) on the 9th post-operative day All animals were preinjected with saline on the 8th day n—number of animals in each group

comparisons was used to evaluate statistical significance of the results Animal groups were taken as an independent measure, treatment (pre-op, post-op 8, post-op 9) and test (pre-swim, post-swim) as repeated measures

RESULTS

The results are presented in Fig 1 Overall analysis of variance revealed a significant effect of animal groups, $F(3,29)=28\ 53$, treatment, $F(2,58)=165\ 8$, and test, $F(1,29)=322\ 59$, all $ps<0\ 001$ The groups×treatment×test interaction was also significant, $F(6,58)=12\ 12$, $p<0\ 001$

The analysis of simple effects showed that pre-swim pain threshold differed neither between animal groups, $F(3,58)=0\ 34$, non-significant—NS, nor between treatments $F(2,116)=0\ 00$, NS The swim produced a pronounced analgesia in the pre-op controls, $F(1,87)=65\ 42$, $p<0\ 001$, which was not group dependent, $F(3,87)=0\ 01$, NS This analgesia remained on the same level throughout the two post-op testings in hormonally intact (SHAM-NaCl) mice, $F(2,58)=0\ 02$, NS but markedly increased after adrenalectomy which was evidenced by a significant simple effect of the group factor on post-op 8/post-swim latencies, $F(3,174)=69\ 03$, $p<0\ 001$

As was found in individual comparisons, dexamethasone preinjection on the post-op 9th day completely blocked the analgesic action of stress in animals with intact adrenals, and reversed the potentiation of this analgesia after adrenalectomy Post-op 9/post-swim hot plate latencies were significantly shorter after dexamethasone than after saline, $t=8\ 79$ $df=174$, $p<0\ 001$ Also, these latencies were shorter with respect to post-op 8/post-swim ones in the SHAM-DEXA and ADREX-DEXA groups, $t=7\ 85$, $df=174$, $p<0\ 001$, but not in two other groups always receiving saline, $t=1\ 25$ $df=174$ NS The post-op 9/post-swim pain threshold in the SHAM-DEXA group fell down to the pre-swim level $t=0\ 83$ $df=174$, NS, whereas in the ADREX-DEXA group, though lowered, was still above the pre-swim values, $t=5\ 12$, $df=174$, $p<0\ 001$

DISCUSSION

Involvement of the pituitary-adrenal axis in creating swim analgesia in mice is especially interesting since its mechanism differs from this one in rats Room temperature water swim (21°C/3 5 min) is completely ineffective to produce analgesia in the rat [5] In order to produce analgesia in this species cold water swim (2, 8, 15°C/3 5 min) is necessary Moreover analgesia induced by room temperature swim (20°C/3 min) in mice is fully suppressed by naloxone at dose of 10 mg/kg [28] while cold water swim analgesia in rats is only partially reversed by dose as high as 20 mg/kg [3] Finding that morphine analgesia in mice, unlike in rats, depends on factors in cerebrospinal fluid [10] makes this comparison additionally interesting

Dexamethasone attenuation of swim induced analgesia in sham operated animals and reversal of its adrenalectomy produced enhancement could indicate involvement of pituitary factor in changing poststress pain sensitivity Since this form of analgesia in mice appears to be mediated by endogenous opioids [28] the most likely one seems to be β-endorphin This endogenous opioid was found to be a potent analgesic when injected intracerebroventricularly [17] and intravenously [25] in mice Moreover, β-endorphin in rats is released from the pituitary concomitantly with ACTH [13,26], its blood level was found to rise after adrenalectomy [13] and to be suppressed after dexamethasone pretreatment [9]

On the other hand, some data strongly argue against such interpretation It was shown that physiological concentration of β-endorphin is much lower than capable of inducing analgesia following intravenous injections [24,25] However Lewis suggested that analgesic concentration of opioids in brain areas responsible for pain inhibition is reached due to

the specific blood circulating system (hypophyseal portal system), or via ventricular system [18] Such interpretation seems to be especially convincing for mice since analgesia producing doses of β-endorphin in this species, however unphysiologically high were found to be much lower than in rats [24,25] Another argument against involvement of pituitary β-endorphin is lack of changes in basal pain sensitivity which should follow adrenalectomy produced pituitary hypersecretion This effect, however, could be explained as a result of functional tolerance which might develop during a relatively long post-surgery period

In conclusion, our data claim for involvement of pituitary opioids in swim analgesia in mice

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