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BRIEF COMMUNICATION

H_2-Receptor-Mediated Stress-Induced Analgesia is Dependent on Neither Pituitary nor Adrenal Activation

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GOGAS, K. R. AND L. B. HOUGH. *H_2-Receptor-mediated stress-induced analgesia is dependent on neither pituitary nor adrenal activation.* PHARMACOL BIOCHEM BEHAV 30(3) 791–794, 1988.—The effects of hypophysectomy and adrenalectomy were studied on the analgesia elicited by a 3 min exposure to 3.5 mA of continuous inescapable footshock, a response previously shown to be resistant to high doses of the opiate antagonist, naloxone, but inhibited by antagonists of histamine H_2-receptors. Neither treatment significantly attenuated the response, and the brain-penetrating H_2-receptor antagonist zolantidine inhibited the response in all surgical treatment groups. These results add further support for our hypothesis that brain histamine and brain H_2-receptors mediate nonopiate analgesia.

Stress Analgesia Histamine H_2-Receptors Opiate receptors Adrenalectomy Hypophysectomy

EXPOSURE to a variety of environmental conditions can induce antinociceptive responses [1, 2, 12, 13]. Pharmacological classification of footshock-induced analgesia (FSIA) has relied extensively on the use of the opiate antagonist naloxone. Inescapable footshock (FS) can produce either naloxone-sensitive (i.e., "opiate"-mediated) or naloxone-insensitive ("nonopiate") analgesia, depending on the temporal pattern and intensity of the FS [6, 7, 13]. Exposure to either intermittent FS (e.g., 3.0 mA over 30 min) or to 3 min of continuous FS at an intensity of 2.0 mA evokes analgesic responses that are blocked by naloxone [6, 7, 13]. Both responses also develop tolerance with daily exposure and show cross-tolerance to morphine [11,14]

The evidence supporting the existence of nonopiate FSIA is equally compelling [6–15], although much less is known about the mediators involved. Two laboratories have confirmed that exposure to 2.0 mA FS for 3 min elicits naloxone-sensitive FSIA and that the analgesia elicited by a 3 min exposure to 2.5 mA is naloxone-resistant, even when the dose of this opiate antagonist in enormous [5, 7, 12, 13]. Administration of alpha-fluoromethylhistidine, an irreversible inhibitor of brain HA synthesis, reduced this naloxone-resistant response [12], suggesting the involvement of the brain transmitter histamine (HA). The response was also inhibited by the HA H_1-receptor antagonist diphenhydramine, but not by the H_2-receptor antagonist cimetidine, implying that H_1- and not H_2-receptors are involved.

Results from our laboratory support and extend these observations [6–10]. Using methods similar to those of Terman *et al.* [13], we confirmed the naloxone-sensitive and naloxone-resistant nature of the FSIA elicited by 2.0 and 2.5 mA, respectively; the lack of activity of H_2-antagonists on the latter response was also confirmed [6,7]. However, exposure for 3 min to a higher current, 3.5 mA, produced analgesia that was inhibited by the H_2-receptor antagonists cimetidine and zolantidine, but not by naloxone [6,7]. Furthermore, no tolerance developed to the analgesia elicited by daily exposure to the 3.5 mA FS paradigm, nor was cross-tolerance between this form of FSIA and morphine observed, supporting the nonopioid nature of this response [15]. These results show the existence of 3 distinct analgesic responses elicited by 3 min of continuous FS of varying intensity: (1) a naloxone-sensitive, zolantidine-insensitive response (2.0 mA), (2) a naloxone-insensitive zolantidine-sensitive response (3.5 mA), and (3) a naloxone-insensitive, zolantidine-insensitive response (2.5 mA). Combinations of naloxone and zolantidine also had no effect on the 2.5 mA response, confirming its non-H_2, nonopiate nature [7].

Classification of environmentally-induced analgesia has also been facilitated by characterization of the effects of hy-

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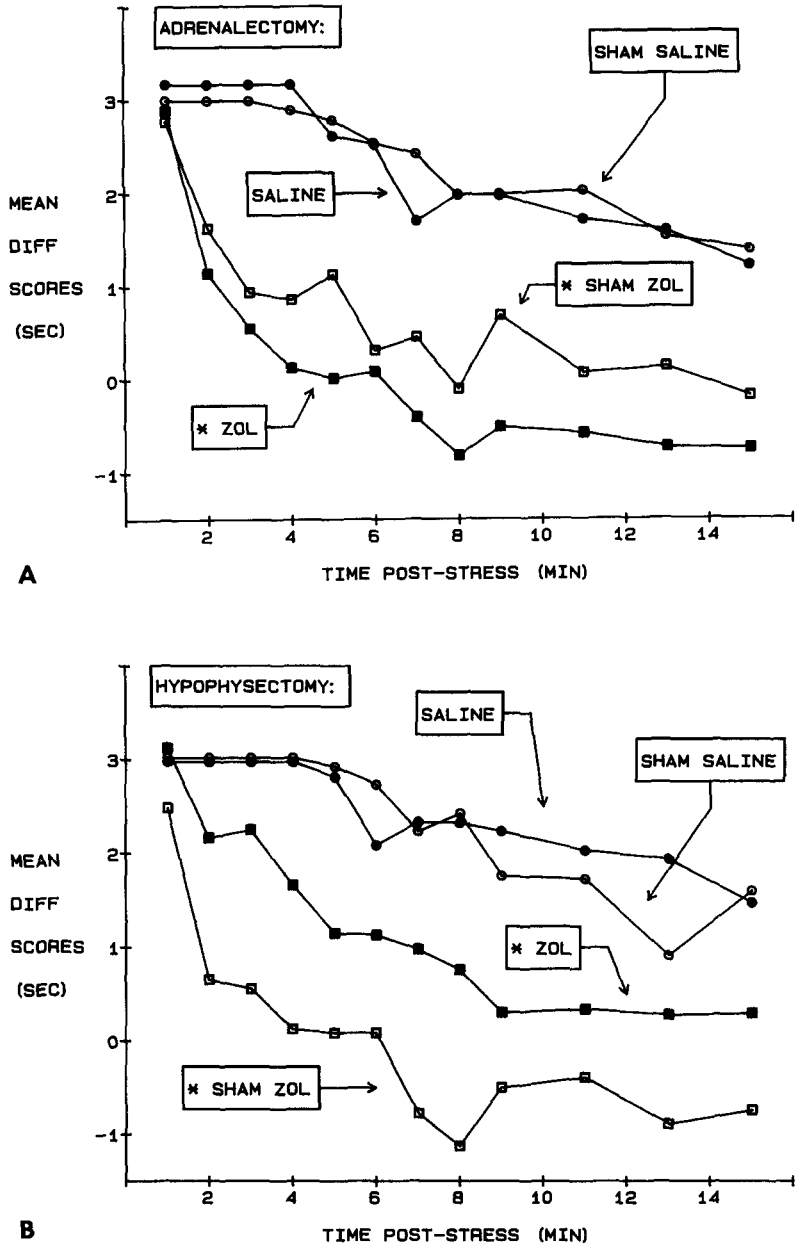

FIG. 1. The effects of adrenalectomy (A, top) and hypophysectomy (B, bottom) on FSIA. Animals received injections of either the H_2-receptor antagonist zolantidine dimaleate (5 mg/kg, SC) or saline vehicle 30 min before poststress testing. They were tested for baseline pain sensitivity, exposed to 3 min of 3.5 mA inescapable FS and then retested at the indicated poststress times (abscissa). Mean analgesic difference scores (poststress latencies minus each animal's prestress baseline) are shown (ordinate) for animals receiving surgery (zolantidine, solid squares, n=6; saline, solid circles, n=6) and sham surgery (zolantidine, open squares, n=6; saline, open circles, n=6). *Indicates groups significantly different from their respective saline control by repeated measures ANOVA ($p<0.001$).

pophysectomy and adrenalectomy on these responses. For example, even though both the intermittent and the continuous FS paradigms induce naloxone-sensitive analgesia (the latter at 2.0 mA), it is clear that these responses are distinct, since the former, but not the latter is reduced by removal of the pituitary as well as the adrenal gland [12,13]. Similarly, the naloxone-resistant analgesia elicited by continuous cold water swims is attenuated by hypophysectomy [2], whereas

the naloxone-resistant, zolantidine-resistant FSIA elicited by 2.5 mA is not [12]. However, the importance of the pituitary or adrenal glands in producing the nonopiate, H_2-mediated 3.5 mA response has not been assessed. Even though our hypothesis is that neuronal HA is released as an analgesic mediator from descending hypothalamic histaminergic neurons, central histaminergic activity has been suggested to modify both pituitary and adrenal function (see [8]). Thus, it seems possible that activation of H_2-receptors could effect analgesia via the release of pituitary and/or adrenal humoral factors. For these reasons, we have presently determined the effect of adrenalectomy and hypophysectomy on the H_2-receptor-mediated FSIA. Since the pharmacological nature of the response could be changed by these surgical treatments without affecting the amount of analgesia expressed, we have also tested the sensitivity of this response to both opiate and H_2-antagonists following surgery.

METHOD

Hypophysectomy and adrenalectomy were performed by the breeder (Taconic Farms, Germantown, NY) on male Sprague-Dawley rats (150–175 g). Hypophysectomy and sham procedures were done using a transaural approach under sodium pentobarbital anesthesia; the completeness of procedure was verified by histological examination of the excised tissue. Bilateral adrenalectomy and sham adrenalectomy were performed under metofane anesthesia. Animals were housed (2/cage) at 27.5°C with free access to food and water on a 12 hr light/dark cycle. Dextrose (5%) and NaCl (1%) served as water for hypophysectomized and adrenalectomized animals, respectively. All behavioral testing was done approximately 10 days following surgery. Pain sensitivity was assessed with a modified version of the radiant heat tail-flick test [3], with a 7 sec upper limit of exposure. The light source was adjusted such that baseline latencies between 3 and 4.5 sec were obtained. Three hr into their dark cycle, animals received blinded injections of either naloxone hydrochloride (10 mg/kg, IP), zolantidine dimaleate (5 mg/kg, SC) or saline vehicle. The intervals between injection and the first poststress test were 10 min for naloxone and its saline vehicle, and 30 min for zolantidine and its saline vehicle. Following injection and before FS, five tail-flick trials were conducted at 1 min intervals with baseline pain sensitivity defined as the mean of the last 3 latencies. Animals were then exposed to inescapable FS for 3 min at 3.5 mA, as described in detail previously [6]. Tail-flick testing resumed 1 min after FS, continued at 1 min intervals until min 9, and thereafter at 2 min intervals until min 15 [6]. Difference scores (obtained by subtracting each animal's mean baseline from each poststress latency) were analyzed by 3-way repeated measures analysis of variance (ANOVA) with the BMDP 2V program [4]. Following testing, all surgical treatments were verified by necropsy.

RESULTS

FS induced a large analgesic response in all vehicle groups. There was no significant difference in the analgesia exhibited by the sham-saline and adrenalectomy-saline animals, indicating that the expression of the analgesia is not dependent on the integrity of the adrenal gland (Fig. 1A). Zolantidine significantly attenuated the FSIA in both the adrenalectomy and the sham groups (Fig. 1A); comparison of the zolantidine curves from these 2 groups showed no significant difference, indicating that the surgery did not alter the ability of zolantidine to block the response. Naloxone also had no effect on the FSIA in either group ($p>0.05$ by ANOVA and Newman-Keuls tests), indicating that the naloxone-insensitivity of the response was maintained following surgery.

Hypophysectomy also failed to affect the magnitude of the FSIA, and zolantidine significantly inhibited the FSIA in both surgical groups (Fig. 1B). However, the analgesia elicited from the hypophysectomy-zolantidine treatment group was significantly greater than that of the sham-zolantidine group (Fig. 1B, $p<0.05$), implying that non-H_2 analgesic mechanisms might also be important in hypophysectomized animals. Naloxone did not inhibit FSIA in either the sham or hypophysectomy group ($p>0.05$ by ANOVA and Newman-Keuls test, data not shown), confirming that the response retained its naloxone-insensitivity (not shown). Neither adrenalectomy nor hypophysectomy had an effect on baseline scores (also not shown).

DISCUSSION

The present results show that neither hypophyseal nor adrenal mechanisms are necessary for the overall expression of the H_2-receptor mediated FSIA. These results add to the evidence suggesting that the response is mediated by neural and not hormonal pathways. The existence of hypothalamic histaminergic neurons projecting to areas such as the periaqueductal grey and spinal cord (see [8]), along with studies showing the analgesic activity of intracerebrally administered HA [5], suggest that these fibers may participate in the modulation of pain transmission.

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