J. Physiol. (1978), **283**, *pp.* 331–340
With 6 text-figures
Printed in Great Britain

THE EFFECT OF PITUITARY-ADRENAL FUNCTION IN THE MODULATION OF PAIN SENSITIVITY IN THE RAT

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(*Received 5 December 1977*)

SUMMARY

1. The relationship between adrenalcortical hormones, adrenocorticotrophic hormone (ACTH), and pain sensitivity was investigated in the rat. Pain sensitivity was assessed by measuring paw-lick and jump latencies in response to being placed on a grid at 55 °C.

2. Bilateral adrenalectomy increased the sensitivity to pain, but adrenal demedullation had no effect.

3. Pain sensitivity was inversely related to the circadian changes in circulating corticosterone and was greater at 7 a.m. than at 7 p.m. However, the same variation in pain sensitivity existed if the adrenals were removed, suggesting that the increase in pain sensitivity after adrenalectomy was not related directly to the levels of corticosteriods.

4. The time course of the increase in pain sensitivity after adrenalectomy paralleled that of the changes in circulating ACTH. Adrenalectomy markedly increased pain sensitivity at 9 and 18 days following surgery when circulating ACTH levels were markedly elevated and corticosterone was absent, but not at 3 days following adrenalectomy when ACTH levels were lower and corticosterone was absent.

5. Hypophysectomy decreased the sensitivity to pain.

6. The results indicate that ACTH can alter pain sensitivity and that the effect of corticosteroids on the sensitivity to pain is an indirect one by virtue of their negative feed-back action on the hypothalamic-pituitary system.

INTRODUCTION

It is well established that hormones exert varied and potent actions on the central nervous system (Vernikos-Danellis, 1972) and that their primary function in the brain is that of neuroregulators or modulators of neural activity (Reith, Schotman, Gispen & De Wied, 1977).

Several studies have suggested that the adrenocortical steroid hormones are involved in mediating sensitivity to sensory stimuli. Detection thresholds for auditory and gustatory stimuli are lowered in humans with primary adrenocortical insufficiency (Henkin, McGlone, Daly & Bartter, 1967; Kosowicz & Pruszewicz, 1966). Similarly, detection thresholds for olfactory stimuli are lowered in humans with the same clinical condition (Henkin & Bartter, 1966) and in the rat following

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adrenalectomy (Sakellaris, 1972). In each of these studies the observed increases in sensitivity associated with subnormal circulating levels of adrenocorticosteroids were returned to normal by treatment with glucocorticoids. The hypothesis was therefore advanced that the adrenocortical steroid hormones, and in particular glucocorticoids, exert a fundamental action on nervous system function with respect to sensory thresholds and that this effect is not specific to any one particular sensory system (Henkin & Bartter, 1966). Although this would imply that alterations in adrenocortical activity might also affect the sensitivity to pain, no such evidence exists in the literature (Gibbs, Sechzer, Smith, Conners & Weiss, 1973).

We undertook the task of investigating the role of endogenous adrenocortical hormones in mediating sensitivity to a pain-producing stimulus in the rat by physiological manipulations of the pituitary-adrenal system *in vivo*. The results indicate that the actions of the adrenocortical hormones in modulating sensitivity to pain are indirect and that the observed effects are primarily due to alterations in the secretion and circulating levels of adrenocorticotrophic hormone (ACTH).

A preliminary account of this work was presented at the Annual Meeting of the Society for Neuroscience, 1977.

METHODS

Subjects. Male adult Sprague-Dawley rats (Simonsen, Gilroy, Ca.) were used. They were housed four or five per cage in plastic top-loading cages with woodchip bedding. Rats had *ad libitum* access to standard laboratory chow and water. Adrenalectomized rats were provided with saline (0·9 % w/v) and hypophysectomized rats were provided with saline (0·9 %) containing dextrose (5 %), instead of drinking water. Rats were kept on a 12 hr light/dark schedule (lights on 7 a.m.) and the temperature was maintained at 23 ± 3 °C. Groups of at least ten rats were used for each experimental condition.

Apparatus. The apparatus employed to assess sensitivity to pain was constructed of clear plastic (Plexiglass). The interior dimensions of this chamber were 24 × 24 × 24 cm. The top of the chamber was mounted on hinges to allow introcution of the rat to the apparatus. The floor of the chamber was constructed from hollow copper tubes. Each tube had an outer diameter of 0·7 cm, and tubes were positioned 0·8 cm apart centre to centre. On the exterior of the chamber, the open ends of these tubes were connected in sequence and attached to a water pump-heater (Haake, Berlin). In this closed system, water was circulated through the tubes of the floor and the desired temperature of the floor could be monitored and maintained within narrow limits.

Procedure. The procedure for estimating pain sensitivity was standardized for all experiments. Following recovery from surgery all rats were initially allowed three 90 sec sessions (one session per day for 3 consecutive days) in the testing chamber with the temperature of the floor maintained at 23 ± 1 °C. This procedure familiarized the rats with handling and allowed for habituation to the novelty of the apparatus. On the fourth and subsequent days the procedure was the same except that the temperature of the floor of the apparatus was maintained at 55 ± 1 °C. If the rats were tested more than once, the tests were conducted on consecutive days. To initiate testing the rat was placed on the grid floor and simultaneously a stop watch was started. Two response latencies were then recorded. The latency to lift from the floor and lick one paw (i.e. paw-lick latency), and subsequently the latency to exhibit a jump response, which consisted of vigorously lifting both hind paws off the grid floor simultaneously (i.e. jump latency), were recorded. The paw-lick and jump latencies were used as indices of sensitivity to the painful stimulus.

During the first experiment, two separate observers recorded latencies for both paw-lick and jump responses. The results between the two observers agreed to within 4 and 7 %, respectively, for the paw-lick and jump response latencies.

During testing the experimenter was not aware of the condition of the rat being tested. If neither a paw-lick nor a jump response was made in 90 sec, the test was terminated and a latency of 90 sec was recorded for each response. Unless otherwise indicated, all testing took place between 7 and 9 a.m.

Surgery. Bilateral adrenalectomy was performed via the dorsal approach under ether anaesthesia. Sham adrenalectomy consisted of only manipulating the adrenal glands bilaterally.

Hypophysectomy was performed under ether anaesthesia via the transauricular approach (Gay, 1967) in a stereotaxic instrument. Sham hypophysectomy consisted of subjecting the rat to the same procedure except that the needle was not inserted into the cranium and the pituitary was not removed. Completeness of hypophysectomy was verified at the time of surgery by examining the removed pituitary, and after sacrifice by exposing and examining sella turcicae.

Bilateral adrenal demedullations were performed under ether anaesthesia via the dorsal approach by exposing the adrenal glands, puncturing the adrenal capsule, and squeezing out the medulla using broad forceps. Sham demedullations were carried out in an identical fashion except that the medulla was not forced out of the gland after puncturing the capsule. Following sacrifice at the completion of testing, completeness of demedullation was verified by embedding each adrenal gland in paraffin, sectioning with a microtome, and staining the mounted sections with haematoxylin and eosin followed by microscopic examination. Data from any rat in which medullary tissue was evident was discarded.

Hormone assays. All blood samples were collected via decapitation following completion of testing. Plasma corticosterone content was determined fluorimetrically. Plasma ACTH content was determined using a conventional 2+1 assay design by the adrenal gland corticosterone content bio-assay in male rats pretreated 4 hr earlier (at 8 a.m.) with prednisolone (5 mg/100 g body wt., s.c.) (Vernikos-Danellis, Anderson & Trigg, 1966). A lyophilized preparation of ACTH (Armour, 50 μ/mg) dissolved in 1 % acetic acid in normal saline was used as the standard. Data were expressed as μg corticosterone/100 ml. plasma and μg corticosterone/mg adrenal tissue or μu. ACTH/ml. plasma.

RESULTS

At 21–23 days following surgery adrenalectomy (n = 10) resulted in reduced paw-lick latencies on all three consecutive tests days compared to the sham adrenalectomized (n = 10) rats ($P < 0{\cdot}01$ each day, Mann–Whitney U test). In addition, adrenalectomy greatly reduced the within-group variability in paw-lick latencies (Fig. 1*A*). Fig. 1*B* shows that adrenalectomy also markedly reduced jump latencies ($P < 0{\cdot}01$ each day), suggesting that the absence of the adrenals leads to increased sensitivity to this painful stimulus.

Due to the fact that adrenalectomy removes a major source of circulating catecholamines as well as corticosteriods, a similar experiment was done in demedullated rats (n = 10). Demedullated rats were tested 21–23 days following surgery when adrenocortical regeneration was complete and corticosterone secretory rates had returned to normal (Buckingham & Hodges, 1975).

Sham demedullated (n = 10) rats served as controls. Plasma corticosterone levels of demedullated rats were not different from those of controls ($P > 0{\cdot}05$) following testing. Fig. 2*A* and *B* show that adrenal demedullation had no effect on either paw-lick or jump latencies.

Since it appeared that the presence of the adrenal cortex was affecting the sensitivity to pain, an experiment was undertaken to determine whether the sensitivity to pain might vary directly with the levels of circulating corticosterone at the time of testing. To test this hypothesis, paw-lick and jump latencies were measured at 21 days following surgery in adrenalectomized and sham adrenalectomized rats at 7 a.m. and 7 p.m. when, in normal rats, the diurnal circulating corticosterone concentration is at its trough and peak, respectively (Krieger, Allen, Rizzo & Krieger, 1971).

Adrenalectomized (n = 20) and sham adrenalectomized (n = 20) rats, at 18–21 days following surgery, were habituated for 3 days and tested for 1 day at either

7 a.m. or 7 p.m. (n = 10/group). Blood samples were obtained 24 hr following testing in order to obtain basal plasma corticosterone levels at these times. The paw-lick and jump latency data were analysed using a 2 × 2 factor (surgical group × testing time) analysis of variance.

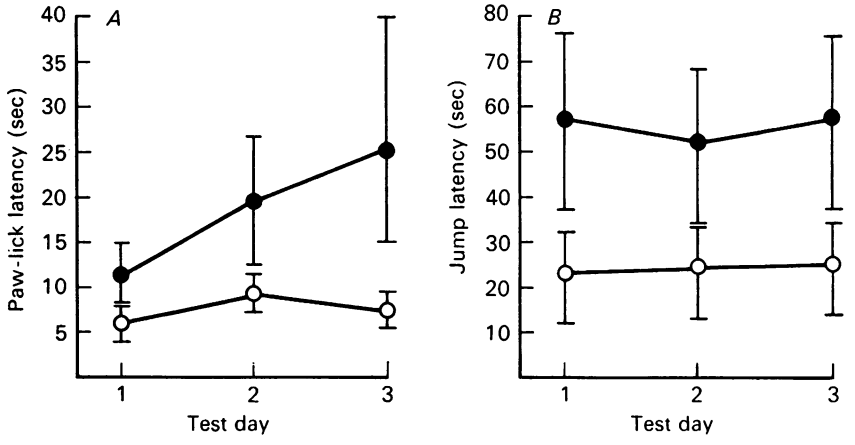

Fig. 1. A and B, effect of adrenalectomy (○——○) and sham adrenalectomy, at 21–23 days following surgery (●——●) on paw-lick (A) and jump (B) latencies to a stimulus of 55 °C on three consecutive test days. Each point represents the mean ± S.E. of mean (n = 10).

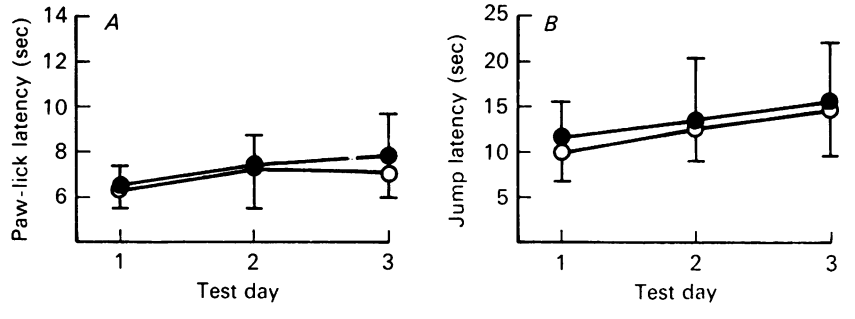

Fig. 2. A and B, effect of adrenal demedullation (○——○) and sham demedullation at 21–23 days following surgery (●——●) on paw-lick (A) and jump (B) latencies. Each point represents the mean ± S.E. of mean.

Pain sensitivity in the sham adrenalectomized groups was greater at 7 a.m., when plasma corticosterone levels were low, than at 7 p.m., when plasma corticosterone levels were high (Fig. 3A and B). Adrenalectomy led to both decreased paw-lick (P < 0·001) and decreased jump latencies (P < 0·001) at both 7 a.m. and 7 p.m. Surprisingly, however, the circadian pattern in pain sensitivity was also present in the adrenalectomized rats. Both paw-lick (P < 0·001) and jump latencies (P < 0·001) were significantly increased at 7 p.m., compared to 7 a.m., regardless of the presence or absence of the adrenal glands. The groups × testing times interaction term in the analysis was not significant, indicating that the response latency lines for the adrenalectomized and sham adrenalectomized rats did not differ significantly from parallelism.

Removal of the adrenal glands not only results in the absence of circulating corticosteroids but also, due to loss of negative feed-back mechanisms, in an increase in blood and pituitary ACTH concentrations (Cox, Hodges & Vernikos, 1958; Fortier, 1959; Hodges & Vernikos-Danellis, 1962). Since the adrenalectomized rats used in these experiments showed increased pain sensitivity when tested at 21–23 days following removal of the adrenal glands when circulating ACTH levels are markedly elevated, it was important to substantiate that this effect was indeed due to increased circulating ACTH by testing animals soon after adrenalectomy when ACTH levels are relatively low but circulating corticosteroids are absent (Cox *et al.* 1958).

Adrenalectomized (n = 30) and sham adrenalectomized rats (n = 30) were habituated and tested for pain sensitivity (n = 10/group) at either 3, 9, or 18 days following surgery. Blood samples were obtained for these rats 4 hr following testing, and plasma ACTH was determined.

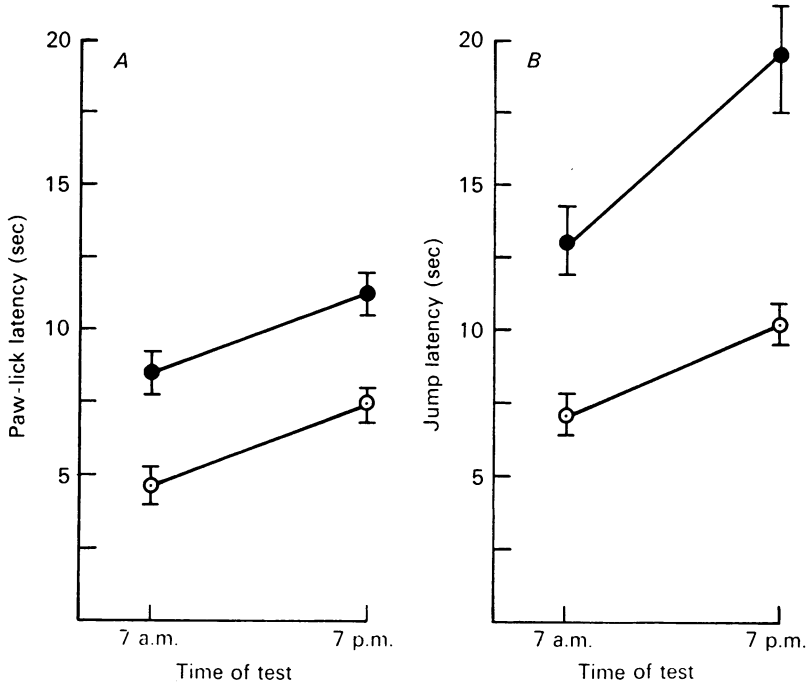

Fig. 3. *A* and *B*, paw-lick (*A*) and jump (*B*) latencies 21 days following surgery in adrenalectomized (○——○) and sham adrenalectomized (●——●) rats at 7 a.m. and 7 p.m., the trough and peak of circulating plasma corticosterone levels, respectively. Each point represents the mean ± S.E. of mean.

Although adrenalectomy led to the expected pattern of increased pain sensitivity as reflected in decreased paw-lick ($P < 0{\cdot}001$) and jump latencies ($P < 0{\cdot}001$) at 9 and 18 days following surgery (see Fig. 4*A* and *B*), at 3 days following surgery the adrenalectomized rats did not show any changes in pain sensitivity, and their response latencies did not differ from the sham adrenalectomized rats.

There was an inverse relationship between the changes in circulating ACTH concentrations and sensitivity to pain in the adrenalectomized rats. Both plasma ACTH levels and pain sensitivity remained unchanged in the sham adrenalectomized controls.

Pain sensitivity was also normal in rats 3 days after removal of the adrenal glands, when plasma ACTH was relatively low, but was markedly greater at 9 and 18 days following surgery, paralleling the increase in circulating ACTH levels (see Fig. 5).

Fig. 6 illustrates the results of an experiment designed to provide further evidence that sensitivity to pain is modulated by the pituitary ACTH secretion rate. Hypophysectomy removes the source of circulating ACTH and should result in reduced pain sensitivity as reflected in longer response latencies.

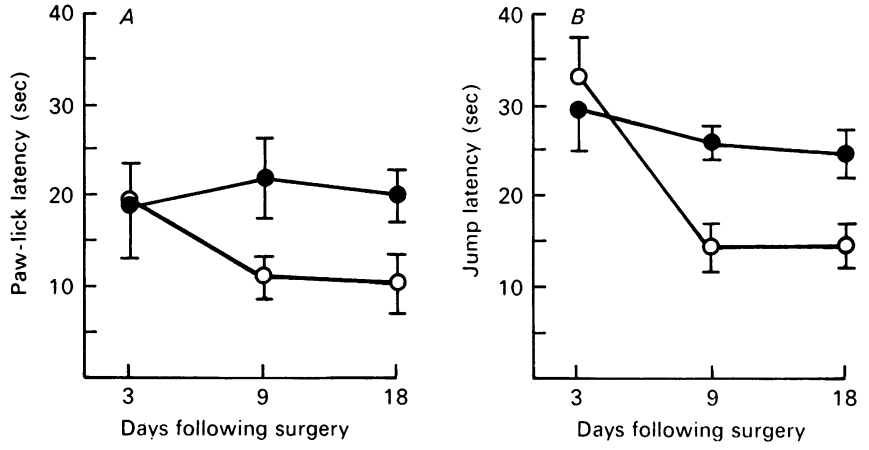

Fig. 4. *A* and *B*, time course of paw-lick (*A*) and jump (*B*) latencies in rats adrenalectomized (○——○) or sham adrenalectomized (●——●) at 3, 9 or 18 days before testing. Each point represents the mean ± S.E. of mean.

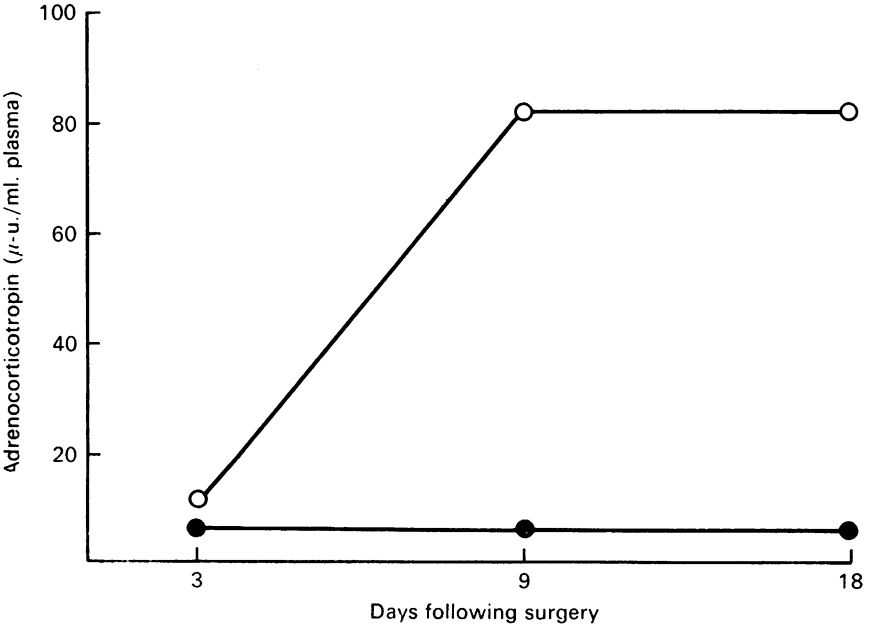

Fig. 5. Plasma ACTH levels in rats adrenalectomized (○——○) or sham adrenalectomized (●——●) at 3, 9 or 18 days previously.

Seven days after hypophysectomy ($n = 14$) or sham phypophysectomy ($n = 10$) rats were habituated to the apparatus for 3 consecutive days and then tested for pain sensitivity on the fourth day. The data were discarded from two rats that were incompletely hypophysectomized.

Removal of the pituitary gland resulted in significantly longer paw-lick ($P < 0{\cdot}001$) and jump latencies ($P < 0{\cdot}05$) when compared to the sham hypophysectomized rats, indicating that hypophysectomy decreases sensitivity to this painful stimulus (see Fig. 6).

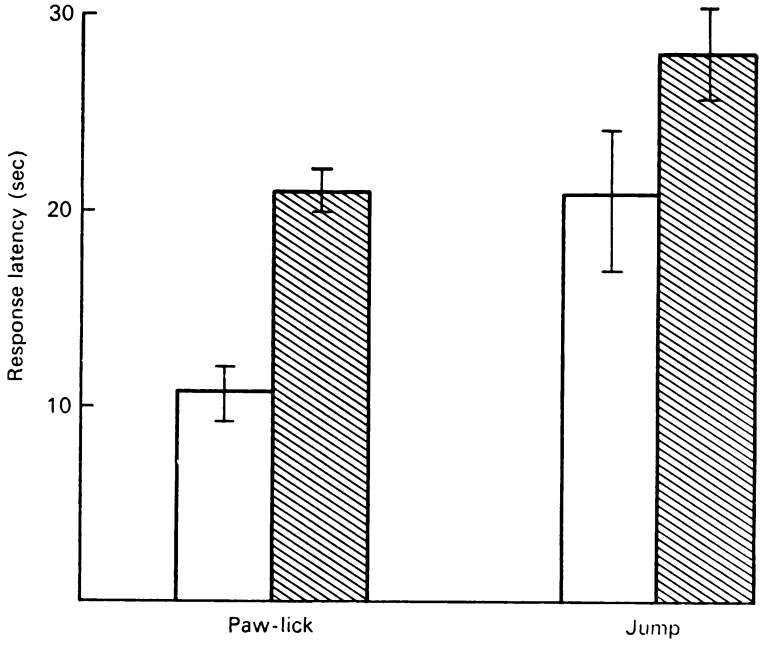

Fig. 6. Paw-lick (left) and jump (right) latencies in hypophysectomized (filled bars) and sham hypophysectomized (open bars) rats. Every column represents the mean ± S.E. of mean.

Throughout this series of experiments it was noted that the jump latencies in the different operated control groups varied between experiments. Although the reason for this variation is unknown, increasing jump latencies were positively correlated with increasing age at the time of testing ($r = +0{\cdot}99$, $P < 0{\cdot}001$). The correlation between paw-lick latencies and age was not significant ($r = +0{\cdot}58$, $P > 0{\cdot}05$).

DISCUSSION

It has generally been assumed that the changes in sensory thresholds accompanying changes in adrenocortical function were due to a direct action of glucocorticoids on the central nervous system (Henkin & Barrter, 1966; Sakellaris, 1972). The present findings indicate that alterations in pain sensitivity were correlated with changes in circulating plasma ACTH levels and provide evidence that ACTH acts as an endocrine neuromodulator involved in pain sensitivity. Our data further suggest that the role hypothesized for adrenocorticosteroids in sensory processes (Henkin & Bartter, 1966;

Sakellaris, 1972) is a secondary one since alterations in circulating corticosteroids affect circulating levels of ACTH via negative feedback mechanisms (Hodges & Vernikos, 1959, 1960).

Two important points in the data support this interpretation exclusively. Sensitivity to pain was not altered 3 days following adrenalectomy when circulating corticosterone was absent and ACTH levels were only slightly elevated. If corticosteroids were the critical endocrine factor involved, increases in sensitivity at 3 days following adrenalectomy would have been expected. Secondly, removal of the pituitary gland led to decreases in sensitivity to pain when, again, the low level of corticosteroids would have been expected to increase sensitivity if corticoids were indeed directly responsible.

Indirect and direct support for this hypothesis of a direct ACTH effect is provided by other lines of investigation. For example, treatment with glucocorticoids, which reduces the abnormally elevated sensitivity to taste, olfaction, and audition in humans with adrenocortical insufficiency and following adrenalectomy in the rat (Henkin & Bartter, 1966; Henkin *et al.* 1967; Kosowicz & Pruszewicz, 1966; Sakellaris, 1972), can also be expected to produce these effects by suppression of the elevated circulating levels of ACTH typically found in these conditions (Sydnor & Sayers, 1954).

More direct support for the hypothesized ACTH effect in increasing pain sensitivity advanced here is provided by the clinical work of Luft & Olivercrona (1955). Hypophysectomy abolished or markedly reduced the pain attendant to advanced breast cancer in nineteen of twenty-four women regardless of whether or not cortisone treatment was continued, suggesting not only a primary role for a pituitary factor but no direct role for glucocorticoids.

In recent years the elegant work of De Wied and his associates has provided considerable evidence that pituitary peptides including ACTH and ACTH-related molecules act as modulators of neural function and are involved in the formation and maintenance of learned behaviours (De Wied, 1966; Greven & De Wied, 1973). In addition it has recently been shown that some ACTH fragments display an affinity for rat brain opiate receptors *in vitro* (Terenius, 1976) and that ACTH fragments, in large doses, partially antagonize the analgesic action of administered morphine (Gispen, Buitelaar, Wiegant, Terenius & De Wied, 1976).

Although the evidence presented here indicates that ACTH is a critical pituitary factor involved in mediating sensitivity to pain, the possibility exists that some other pituitary peptide might be responsible. The opioid peptide β-endorphin, as well as β-lipotrophic hormone (β-LPH), the common precursor for both ACTH and β-endorphin (Mains, Eipper & Ling, 1977), appear to be located in the same anterior pituitary cells that contain ACTH (Pelletier, Leclerc, Labrie, Cote, Chretien & Lis, 1977). The release of β-LPH from the pituitary has been shown to parallel that of ACTH (Abe, Nicholson, Liddle, Orth & Island, 1969; Gilkes, Bloomfield, Scott, Lowry, Ratcliffe, Landon & Rees, 1975) and recently it was reported that pituitary ACTH and β-endorphin secretion are under the control of a common feedback regulatory mechanism (Guillemin, Vargo, Rossier, Minick, Ling, Rivier, Vale & Bloom, 1977). The question then arises as to whether alterations in the levels of these circulating peptides, concurrent with altered ACTH levels, might be responsible for the observed effects on pain sensitivity. This possibility is unlikely. β-endorphin is

an opioid peptide and, as such, increased circulating levels as a result of adrenalectomy (Guillemin *et al.* 1977) would be expected to produce analgesia. Peripheral injections of β-endorphin do indeed produce analgesia in the mouse (Tseng, Loh & Li, 1976). Conversely, hypophysectomy and the resulting decrease in circulating β-endorphin (Guillemin *et al.* 1977) would be expected to increase pain sensitivity. In fact, the opposite is true; hypophysectomy in both our studies and in humans (Luft & Olivecrona, 1955) reduces pain sensitivity and adrenalectomy increases it. We therefore propose that ACTH is the critical pituitary peptide modulating the alterations in pain sensitivity observed here. However, it should also be noted that hypothalamic-median eminence corticotropin-releasing activity increases as a function of time following adrenalectomy (Vernikos-Danellis, 1965). A possible role of this peptide cannot be discounted.

Although the mechanism of this neuromodulatory action are unknown, it is possible that ACTH may serve as an endogenous opiod antagonist. ACTH, and β-endorphin might act antagonistically on the function of a common central peptidergic neural system. The proposed central enkephalinergic system (Goldstein, 1976) or the putative substance P containing primary afferent fibres projecting to the dorsolateral horn of the spinal cord (Takahashi, Konishi, Powell, Leeman & Otsuka, 1974) or substance P systems in brain (Brownstein, Mroz, Kizer, Palkovits & Leeman, 1976) are likely candidates as the target systems for the interaction of ACTH with pain perception mechanisms.

We would like to thank Armour Laboratories, Inc. (Kankakee, Ill.) for its generous gifts of ACTH, and also Carol Gonzales for her technical assistance.

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