Recent Progress in HORMONE RESEARCH

The Proceedings of the Laurentian Hormone Conference

Edited by
GREGORY PINCUS

VOLUME X

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PREFACE

The tenth annual meeting of the Laurentian Hormone Conference took place in September, 1953, at Mont Tremblant, Quebec. The Committee on Arrangements is indebted to the following contributors whose financial assistance made possible the holding of the Conference: Abbott Laboratories, The Armour Laboratories; Ayerst, McKenna & Harrison, Ltd.; Baxter Laboratories; Carroll Dunham Smith Pharmacal Co.; Chemical Specialties Co., Inc.; Ciba Co., Ltd.; Ciba Pharmaceutical Products, Inc.; Endo Products, Inc.; Charles E. Frosst & Co.; The Glidden Co.; Hoffmann-La Roche, Inc.; Frank W. Horner, Ltd.; Lederle Laboratories Division; Eli Lilly & Co.; Mallinckrodt Chemical Works; Merck & Co., Inc.; The Wm. S. Merrell Co.; Nordic Biochemicals Ltd.; Organon, Inc.; Ortho Research Foundation; Parke, Davis & Co.; Schering Corporation; Chas. Pfizer & Co., Inc.; Schieffelin & Co.; G. D. Searle & Co.; Sharp & Dohme, Inc.; Smith, Kline & French Laboratories; The Squibb Institute for Medical Research; Sterling-Winthrop Research Institute; Syntex, S. A.; The Upjohn Co.; Warner-Chilcott Laboratories, Inc.; The Wilson Laboratories; Wyeth, Inc. Their generosity made it possible to have as special guests from abroad Dr. R. Pitt-Rivers of the National Institute for Medical Research of London, England, Dr. Bernhard Zondek of the Rothschild Hadassah University Hospital, Jerusalem, Israel, and Dr. Rolf Luft of the Serafimerlasarettet, Stockholm, Sweden.

The Committee is also grateful to the chairman of the various meeting sessions: Drs. E. Anderson, R. Rawson, R. Dorfman, E. B. Astwood, J. Leathem, I. T. Nathanson, and J. Jailer. Their leadership has been responsible for the active, critical discussions characteristic of the Conference. Miss Joanne Sanford, Miss Natalie Raymond, and Miss Ruth Kirby gave invaluable secretarial assistance to the Committee, and Mrs. L. P. Romanoff has been especially helpful in the preparation of the index.

A more than usual representation of members from abroad was a special feature of this meeting. To all attending, the international character of scientific inquiry and investigation was indubitably apparent. The spirit of the Conference was reenforced. If it is reflected again in this volume the meeting's purpose is attained.

GREGORY PINCUS

Shrewsbury, Massachusetts

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I. NERVOUS SYSTEM — HORMONE INTERRELATIONSHIPS

The Central Nervous System and Stress-Induced Eosinopenia*

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The role of the central nervous system in the regulation of anterior pituitary function is incompletely understood. It is well-known that many types of stress stimuli will evoke an increase in the secretion of ACTH. Many of these environmental factors are perceived only by exteroceptive or special sensory organs and are hence mediated through the nervous system. Moreover, even emotional factors associated with impending stress are often sufficient to effect hormonal changes. However, the exact portions of the brain which are involved and the manner in which the stimulus is conveyed to the adenohypophysis are far from clear. It has been suggested that the central nervous system might stimulate ACTH secretion through autonomic discharge, with the subsequent elaboration of epinephrine by the adrenal medulla which would then act directly upon the adenohypophysis (9). Other investigators feel that the brain participates in a more direct manner by the elaboration of a humoral substance in the hypothalamic region which stimulates the function of the anterior pituitary gland (7, 8). Certainly, direct neural connections concerned with secretomotor function of the adenohypophysis have never been demonstrated conclusively.

This investigation was designed to study neural activity related to stress-evoking stimuli and to determine if possible the exact regions of the brain which are capable of influencing the response to these stimuli. Many of these experiments were performed first in cats and have been reported previously (13, 14). It was shown that the electrical activity of the posterior aspect of the hypothalamus could be altered by the administration of certain stress stimuli, epinephrine in particular. Furthermore, it was demonstrated that destruction of this same area prevented the characteristic eosinopenic response to subsequently administered stress stimuli. Electrical excitation of this region of the hypothalamus produced a significant fall in the level of circulating eosinophilic leucocytes in the blood. It was concluded

* Aided by grants from the National Institutes of Health, U. S. Public Health Service and the Commonwealth Fund.

that the hypothalamus was intimately concerned with the transmission of acute stress stimuli to the adenohypophysis in the cat. The present work was undertaken to see if a similar conclusion might be valid in primates and to explore further the interrelationships between the nervous and endocrine systems in the control of ACTH secretion.

Macaca mulatta monkeys were used for this investigation. Most animals prepared for acute experiments were anesthetized with cyclopropane anesthesia, as this agent did not in itself appreciably affect the eosinophil count. Furthermore, it did not inhibit the eosinopenic response to subsequent stimuli. Local anesthesia at operative sites was effected with 2% procaine. Alterations in the level of circulating eosinophilic leucocytes in the blood was used as an index of stress (16). It is readily admitted that this test is not a specific index, although it is certainly very sensitive to increased adrenal cortical function. However, in our preliminary experiments, it was demonstrated that the stress stimuli employed did not evoke a significant eosinopenia in the absence of the adrenal glands under the conditions of these experiments. It was adopted, therefore, as a guide to adrenal cortical function for acute experiments with the realization that the results should be confirmed by other indices when such are practical for this animal.

I. Electrical Recording Experiments

The first investigative approach to the problem of neural involvement in the response to stress stimuli was designed to study electrical changes in the brain as an index of its activity. Increased electrical activity of the brain induced by epinephrine and other autonomic drugs was first reported by Grinker and Serota (6). They made these observations by the use of pharyngeal and scalp electrodes and implicated both the hypothalamus and the cerebral cortex. More recent work of Simms, Pfeiffenberger, and Heinbecker, has shown increased activity in the cerebral cortex alone (18). In the present study, electrodes were placed throughout the brain stem and cerebral cortex in an attempt to localize more precisely those regions which exhibited altered electrical activity in response to stress stimuli. In addition, a more extensive analysis of the conditions under which such changes might be encountered was undertaken. Electrodes were stereotaxically placed in the brain and records of the electrical activity were obtained with a Grass amplifier and inkwriter. Stimuli were then applied which had been found to evoke a significant fall in the level of circulating eosinophils.

A definite increase in the electrical activity of the hypothalamus could consistently be induced by epinephrine (Fig. 1). As previously reported in cats, this was reflected in both the amplitude and frequency of the response (13). Such activity began approximately 1 minute after complet-

ing the intravenous injection of epinephrine (5-15 μg.) and reached a maximum within 2 to 3 minutes. At the time of maximal discharge, the amplitude was often twice that of the spontaneous activity. The frequency increased from the normal range of 5 to 10 waves per second to 15 to 18 per second. This is somewhat faster than that seen in the cat. Normal

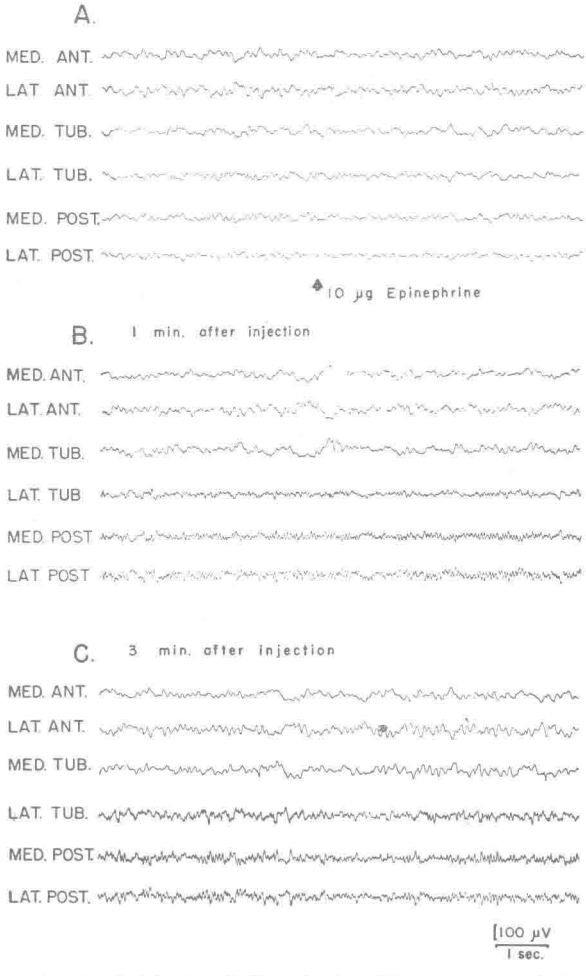

FIG. 1. Records of electrical activity of six different areas of the hypothalamus showing effect of intravenously administered epinephrine (A). Increased neural discharge is seen in lateral tuberal and posterior regions beginning 1 minute after injection (B), and reaching a maximum within 3 minutes (C). Anterior and medial tuberal areas are unchanged.

activity was usually restored within 5 minutes, although in some cases augmented electrical discharge was evident for as long as 15 minutes after injection. Similar activity could be evoked by intravenous insulin (0.5 unit/kg.) and hypoxia. With insulin, the onset of the discharge occurred approximately 1 to 3 minutes after the injection was completed, but otherwise was indistinguishable from that obtained with epinephrine. An almost identical response was obtained with hypoxia beginning about 3 minutes after the oxygen supply had been removed and nitrogen substituted.

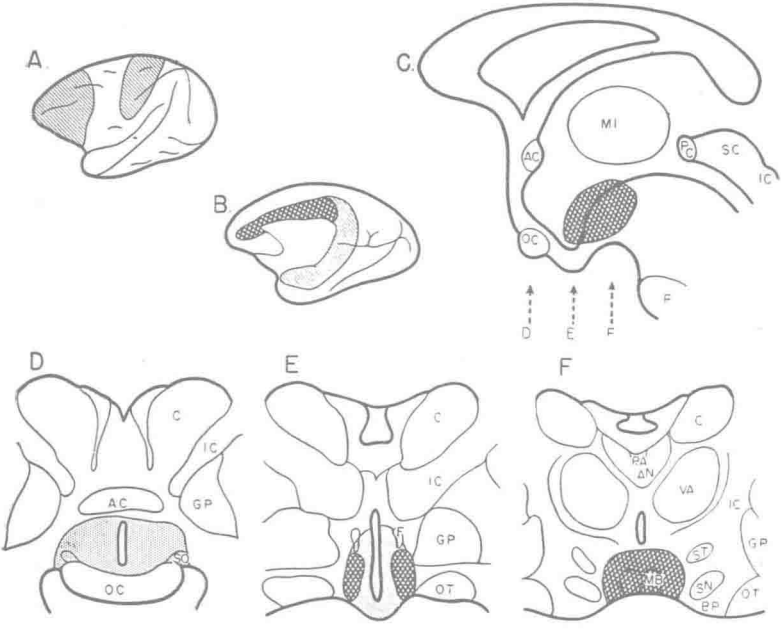

FIG. 2. Outline of lateral (A) and medial (B) surfaces of cerebral cortex and midsagittal reconstruction of brain stem of monkey with transverse sections at levels indicated by arrows (D, E, and F). Regions which exhibited marked changes in electrical activity after the intravenous injection of epinephrine are indicated by cross-hatching. Areas in which only minor changes could be detected are shown by cross-lining. Other areas failed to show any alterations. Negative regions in the hypothalamus are specifically indicated by stippling.

The regions of the brain in which these changes could consistently be demonstrated were quite specific (Fig. 2). In the brain stem, altered electrical activity was encountered only in the posterior and tuberal regions. Such discharges were limited to the lateral hypothalamic area at the tuberal and posterior levels, the mammillary bodies and the region just dorsal to

them. Negative results were obtained in the median eminence. Other regions of the brain stem were thoroughly explored, and with only one exception no such activity could be demonstrated. The anterior nucleus of the thalamus was found to display augmented activity paralleling that seen in the hypothalamus. This thalamic discharge was shown to be relayed from the hypothalamus.

An increase in the electrical activity of certain regions of the cerebral cortex was also observed. The anterior cingulate gyrus showed a marked degree of augmented electrical activity on injection of epinephrine which closely followed that seen in the hypothalamus. Similar changes, but to a lesser degree, were observed in the posterior cingulate and hippocampal gyri. Minor alterations were evident in the somatic sensory and prefrontal cortex also (Fig. 2).

In several preparations the cerebral cortex was removed in its entirety. Under such conditions there was no significant alteration in the epinephrine-induced hypothalamic discharge observed in intact animals. In other experiments augmented neural discharge was again noted in the hypothalamus after sectioning the neuraxis up to the level of the mesencephalon. The character of the discharge was altered somewhat, however, in that as ascending sections were made in the brain stem, the frequency of the discharge increased.

On the other hand, augmented activity seen in the cortex was dependent upon an intact hypothalamus and was not caused by a direct humoral action. This was shown by making electrolytic lesions in the hypothalamus and observing no change in the spontaneous electrical activity in the cortex on injecting epinephrine into the blood.

The possibility that such a hypothalamic discharge might be secondary to the augmented secretion of anterior pituitary or adrenal cortical hormones brought about by stress stimuli was investigated. Adrenocorticotrophic hormone (0.5 to 20.0 units) was injected intravenously while tracings of the electrical activity of the brain were being made. In no instance, however, could an increased electrical activity be observed in the brain stem up to an hour after injection. In fact, with dosages of 5 units or over, a suppression of spontaneous discharge of the anterior brain stem could be demonstrated (Fig. 3). This began from 15 to 20 minutes after injection and lasted for 5 to 10 minutes, following which normal activity was restored. This diminished discharge was manifested largely by a decrease in amplitude, but to a certain extent by a decrease in frequency, also. If, during this period of suppressed activity, epinephrine was injected, no augmented electrical discharge was produced (Fig. 3). When ACTH was injected in dosages of less than 5 units no suppression of spontaneous discharge was demonstrable,

but still the epinephrine-induced discharge previously seen was prevented. This effect was not a direct one of ACTH for in the absence of the adrenal glands no such suppression was observed. Furthermore, the injection of aqueous adrenal cortical extract (Upjohn, 2-10 cc.) produced similar alterations in the electrical activity of the hypothalamus (Fig. 4). However, suppression of neural discharge could not be produced with 2 to 20 mg. of cortisone acetate (Fig. 5), hydrocortisone acetate, or desoxycorticosterone

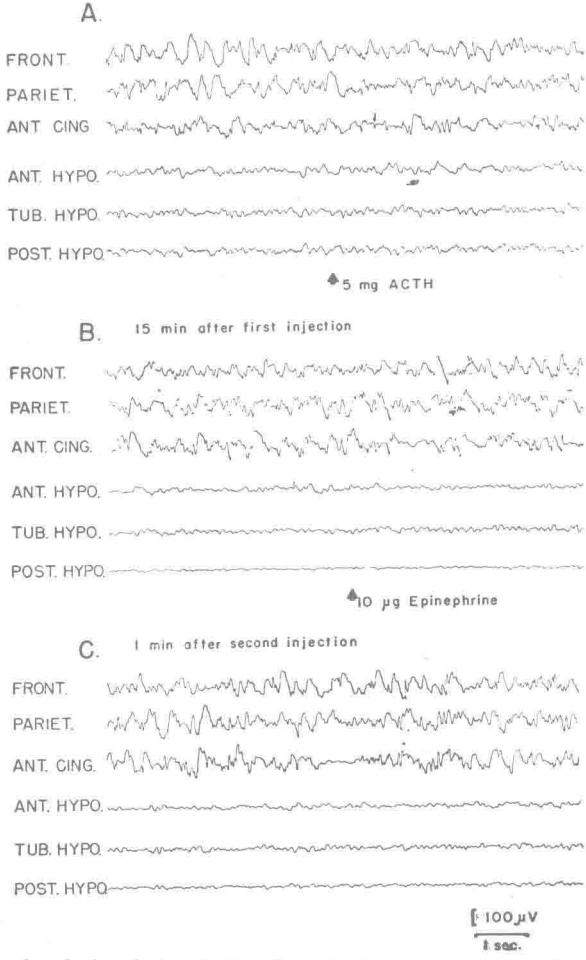

FIG. 3. Records of electrical activity of cerebral cortex and hypothalamus showing effect of intravenously administered ACTH. Suppression of anterior brain stem discharge is noted (B) within 15 minutes after injection (A). Subsequently injected epinephrine (B) failed to evoke the increased hypothalamic discharge seen in Fig. 1 (C).

acetate (Fig. 6). The administration of these three steroids did not alter epinephrine-induced discharge in the hypothalamus. Changes in the activity of the cerebral cortex were noted with the adrenal cortical hormones as had been previously reported (5).

Although the adrenal cortical steroids did not increase the neural activity of the hypothalamus, their essentiality for epinephrine-induced discharge

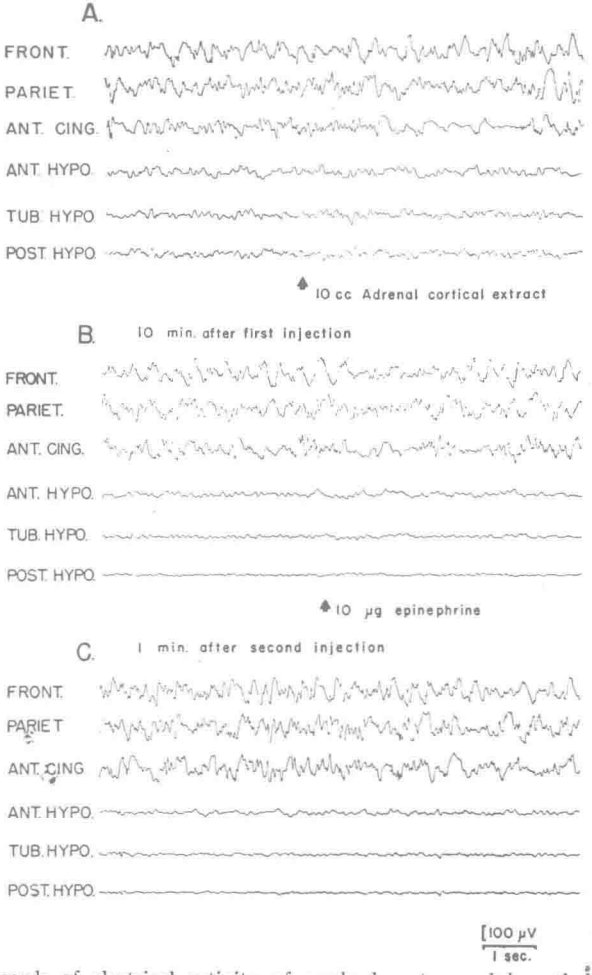

FIG. 4. Records of electrical activity of cerebral cortex and hypothalamus showing effect of intravenously administered adrenal cortical extract. Suppression of anterior brain stem discharge is noted (B) within 10 minutes after injecton (A). Subsequently injected epinephrine (B) failed to evoke the increased hypothalamic discharge seen in Fig. 1 (C).

was studied. Animals were totally adrenalectomized and maintained for 48 hours without replacement therapy. The responsiveness of the hypothalamus to epinephrine was then tested. Under these conditions, however, there was no augmented electrical activity observed, even with ten times the amount of epinephrine. The normal response to epinephrine could be readily restored by the administration of a minimum of 0.5 mg.

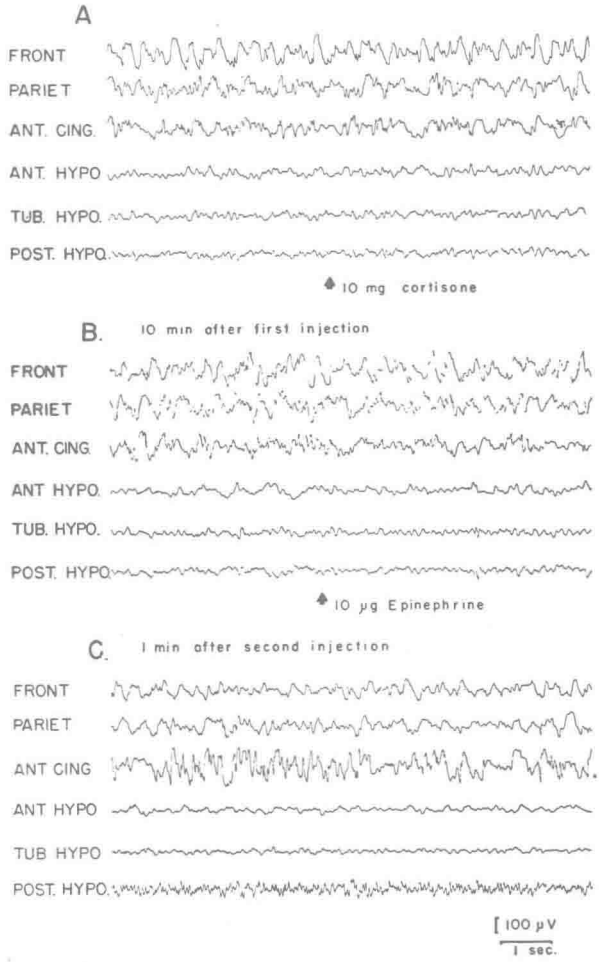

FIG. 5. Records of electrical activity of cerebral cortex and hypothalamus showing effect of intravenously administered cortisone acetate. No change is noted in the neural discharge of the hypothalamus (B) 10 minutes after injection (A). Subsequently administered epinephrine (B) evoked the previously noted augmented electrical activity of the hypothalamus (C).

of cortisone (Fig. 7). Hydrocortisone had the same effect, although desoxycorticosterone did not.

In the light of these observations, it appears that certain stimuli which can induce an eosinopenia are also capable of altering the electrical activity of the hypothalamus. Whether this neural discharge is actually concerned with the transmission of the stimulus to the anterior pituitary gland as

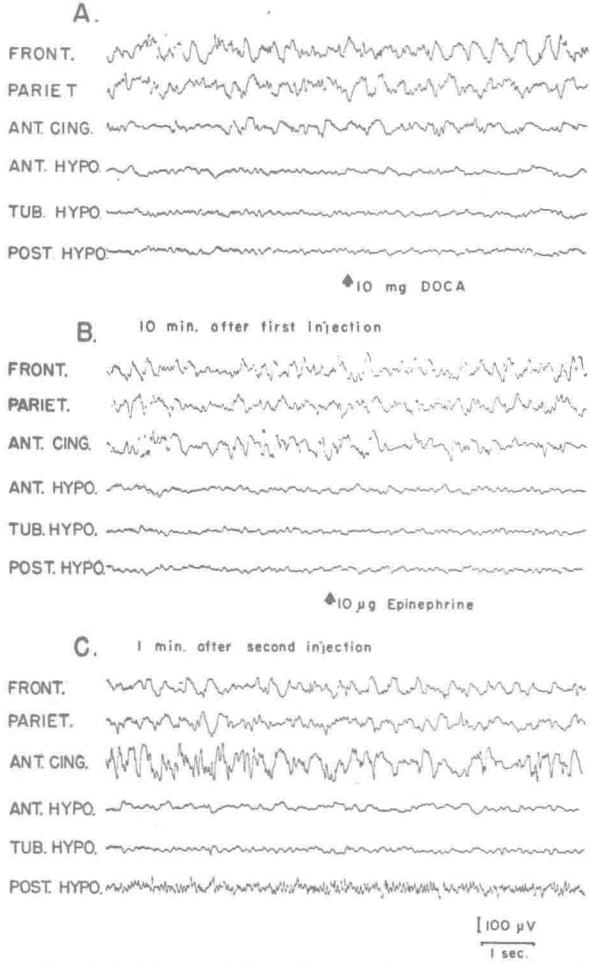

FIG. 6. Records of electrical activity of cerebral cortex and hypothalamus showing effect of intravenously administered desoxycorticosterone acetate. No change is noted in the neural discharge of the hypothalamus (B) 10 minutes after injection (A). Subsequently administered epinephrine (B) evoked the previously noted augmented electrical activity of the hypothalamus (C).

found in the cat, or is merely an unrelated event, cannot be determined by these experiments alone. It seems likely that the augmented activity is not the result of the increased secretion of ACTH or adrenal cortical steroids, as the administration of these hormones failed to evoke such a change. The

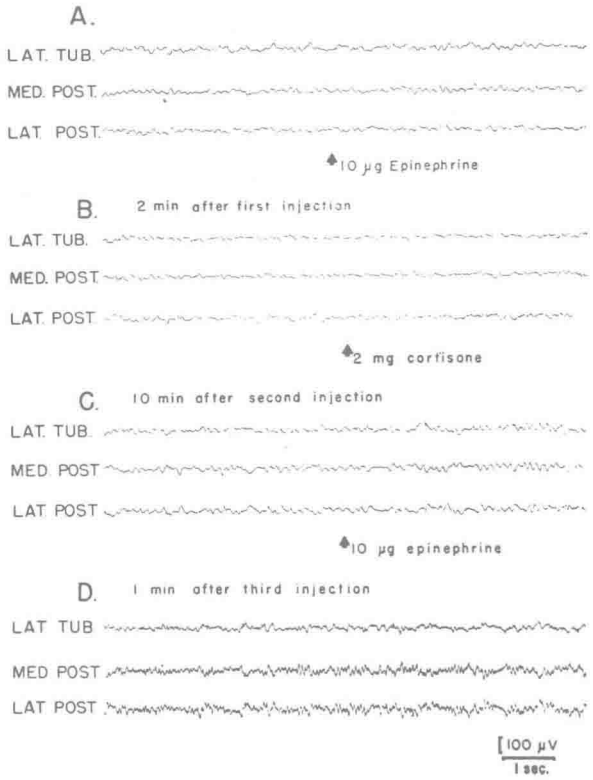

FIG. 7. Records of electrical activity of hypothalamus showing no increase in the electrical activity of the hypothalamus (B) after the intravenous administration of epinephrine (A) 48 hours after bilateral adrenalectomy. Epinephrine was again injected (C) 10 minutes after the intravenous administration of cortisone (B) and the previously noted hypothalamic discharge was evoked (D).

presence of adrenal cortical hormones, however, was shown to be a necessary concomitant for the epinephrine-induced neural discharge.

Epinephrine-induced neural discharge in other regions of the brain appears to be an indirect effect dependent upon activation of the hypothalamus. At least this is the case at this dosage of epinephrine. The most pronounced effect outside of the hypothalamus was seen in the anterior cingulate gyrus. The well-established anatomical pathway from the mammillary bodies by