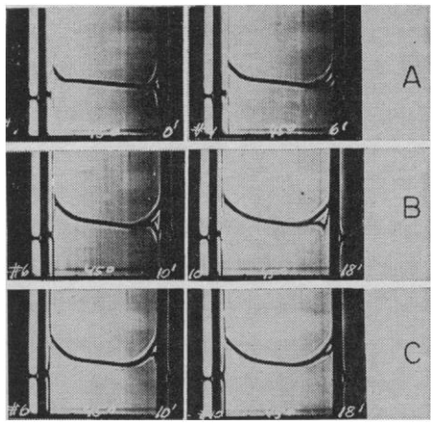

Fig. 2. Analytical ultracentrifuge photographs of the three lipoprotein components separated from plasma membranes treated with sonic oscillation followed by fractionation in KBr gradients. Movement of boundaries is from right to left, in the centripetal direction. Speed was 31,410 rev/min in a double-sectored cell. Schlieren diaphragm angle is 45° for all pictures taken at different times (0, 6, 10, and 18 minutes) after a speed of 31,410 rev/min was attained.

less rapidly and that floated more slowly than did the component shown in Fig. 2A. The total boundary was seen after 16 to 18 minutes of centrifugation at 31,410 rev/min. This class had an S_f of $9.1_{(1.155)}$.

Figure 2C shows the class of lipoproteins obtained after preparative centrifugation of the infranatant from above, at a solution density of 1.210 g/ml. The final solution density was 1.193 g/ml, and the lipoprotein had an S_f of $5.1_{(1.193)}$. Recentrifugation of solutions containing the lipoproteins caused no visible alterations in the boundaries at any of the densities.

We dialyzed the final infranatant solution ($d = 1.210$ g of KBr per milliliter) against 0.15*M* NaCl to remove the large amount of KBr. Only one sedimenting component was observed in the analytical ultracentrifuge; its sedimentation coefficient was 2.6. When the infranatant was dialyzed against phosphate-NaCl buffer (*p*H 7.5, ionic strength 0.20), only one sharp boundary was obtained in moving-boundary electrophoresis. It had a mobility of -4.87×10^{-5} cm^2 volt^{-1} sec^{-1}.

Isolated plasma membranes, not treated with sonic oscillation, routinely contained 25 percent protein that was soluble in 0.15*M* NaCl and that had the same sedimentation coefficient (2.6) as the single protein remaining in the ultracentrifugal residue ($d = > 1.21$ g/ml) from membranes treated with sonic oscillation (SS). The remaining 75 percent of protein from untreated membranes was best solubilized by 1 percent sodium dodecylsulfate. When this protein was subjected to ultracentrifugation, three sedimenting components were resolved during the sedimentation procedure.

Lipoprotein macromolecules can be isolated from plasma membranes of liver from the rat, when suitable, meticulous procedures are employed. No low-density lipoproteins (those that would float in solution density of < 1.0635 g/ml) were ever observed. All the lipoproteins isolated were high-density and had different ratios of lipid to protein. Current experiments suggest that those lipoproteins floating in solution density of 1.125 g/ml have a higher ratio of lipid to protein than the other two classes.

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References and Notes

1. J. D. Robertson, *Ann. N.Y. Acad. Sci.* **137**, 421 (1966); J. F. Danielli and H. Davson, *J. Cell. Comp. Physiol.* **5**, 495 (1935).
2. J. De Gier and L. L. M. Van Deenen, *Biochim. Biophys. Acta* **49**, 286 (1961).
3. L. L. M. Van Deenen, in *Progress in the Chemistry of Fats and Other Lipids*, R. T. Holman, Ed. (Pergamon Press, London, 1965), part 1, vol. 8, p. 1.
4. D. E. Green and J. F. Perdue, *Proc. Nat. Acad. Sci.* **55**, 1295 (1966).
5. J. W. Gofman, F. T. Lindgren, H. Elliott, *J. Biol. Chem.* **179**, 973 (1949).
6. P. Emmelot, C. J. Bos, E. L. Benedetti, PH. Rumke, *Biochim. Biophys. Acta* **90**, 126 (1964).
7. V. P. Skipski, M. Barclay, F. M. Archibald, O. Terebus-Kekish, E. S. Reichman, J. J. Good, *Life Sci.* **4**, 1673 (1965).
8. M. Barclay, V. P. Skipski, O. Terebus-Kekish, R. K. Barclay, *Fed. Proc.* **25**, 656 (1966).
9. D. M. Neville, Jr., *J. Biophys. Biochem. Cytol.* **8**, 413 (1960).
10. D. F. H. Wallach and V. B. Kamat, in *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1966), vol. 8, p. 164; P. Emmelot and C. J. Bos, *Biochim. Biophys. Acta* **120**, 369 (1966).
11. S. J. Cooperstein and A. Lazarow, *J. Biol. Chem.* **189**, 665 (1951).
12. C. W. Tabor, H. Tabor, S. M. Rosenthal, *ibid.* **208**, 645 (1954); C. Schnaitman, V. G. Erwin, J. W. Greenawalt, *J. Cell Biol.* **32**, 719 (1967).
13. S. Ochoa, in *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1955)), vol. 1, p. 735; A. I. Caplan and J. W. Greenawalt, *J. Cell Biol.* **31**, 455 (1966).
14. M. A. Swanson, in *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1955), vol. 2, p. 541.
15. M. G. Farquhar and G. E. Palade, *J. Cell Biol.* **26**, 263 (1965).
16. Supported in part by NCI grant CA-08748. We thank Mrs. Mary D. Sichel and Mr. Edward M. Greene for their generous assistance.

27 January 1967; revised 27 March 1967

Steroid-Sensitive Single Neurons in Rat Hypothalamus and Midbrain: Identification by Microelectrophoresis

Abstract. *Minute amounts of Na-dexamethasone-21-phosphate administered by microelectrophoresis to the immediate extracellular environment promptly suppressed electrical activity of 15 out of 115 hypothalamic and mesencephalic neurons, the effect being readily reversible. Such neurons marked with fast green were found to lie in circumscribed areas of the periventricular gray of the third ventricle and aqueduct, and may represent a site of action of adrenocortical steroids in the regulation of corticotrophin releasing factor and/or adrenocorticotropin secretion by negative feedback.*

Studies using electrical stimulation (*1*), placement of electrolytic lesions (*2*), pituitary transplants (*3*), and especially implantation of steroid cristals (*4*) have implicated several hypothalamic and midbrain structures in the negative feedback exerted by adrenocortical steroids upon the synthesis and/or release of adrenocorticotropin (*5*). In particular, the basomedial hypothalamus (nuclei arcuatus and ventromedialis) and the mesencephalic reticular formation seem to be involved. While the results so obtained are remarkably consistent if species variations and limitations inherent in these experimental approaches are taken into account, there is still no agreement on the *exact* location of the presumed cerebral steroid receptors. Furthermore, little information is available on the kinetics of these feedback reactions, most experiments being of necessity carried out in chronic preparations which do not necessarily reflect short-term physiological interactions. The use of more refined techniques operating on the level of the single neuron (*6*, *7*) therefore appears to be in order.

The technique of microelectrophore-

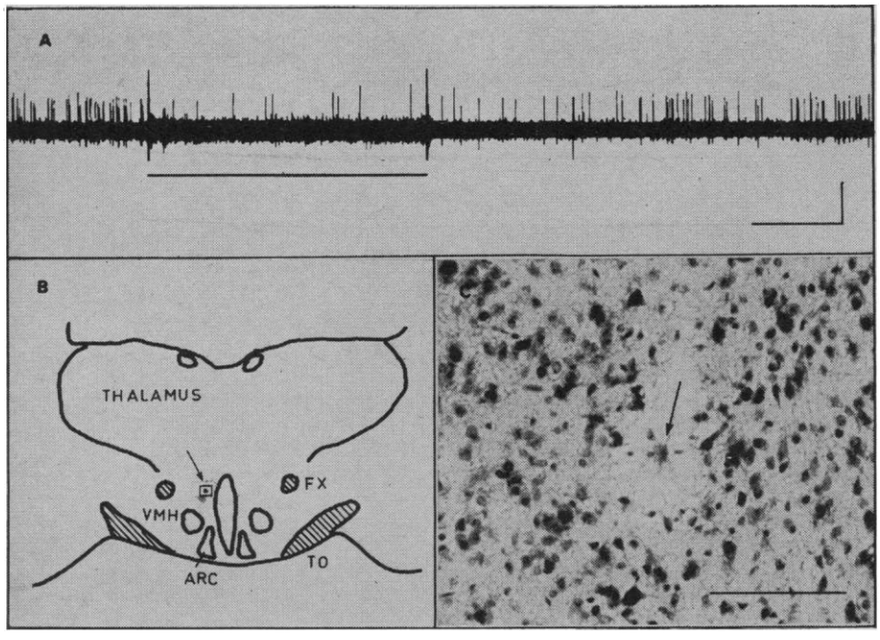

Fig. 1. (A) Inhibition of the spontaneous activity of a single neuron in the anterior hypothalamus by microelectrophoresis (——) of dexamethasone (20 na) (noise level reduced by 60 percent). Calibration: horizontal bar, 10 seconds; vertical bar, 0.3 mv. (B) Gross localization of recording point (arrow) from which response shown in (A) was obtained. The section corresponds approximately to the frontal plane 6.6 of the atlas of Albe-Fessard *et al.* (*15*). The rectangular field refers to (C). *FX*, fornix; *TO*, tractus opticus; *VMH*, nucleus ventromedialis hypothalami; *ARC*, nucleus arcuatus. (C) Fine localization (arrow) of the electrode tip by means of the fast green technique (microphotograph; scale line 100 μ).

sis (*8*), developed over the past years for neurophysiological and for neuropharmacological studies, is especially suited for this type of neuroendocrine research. Its main advantage is the possibility of delivering, from multibarreled micropipettes, minute amounts of drugs (for example, hormones) into the immediate extracellular environment of single neurons and observing the response of these cells directly and immediately in terms of changes in the discharge rate of action potentials. Many drugs can thus be applied in small but efficient concentrations (in the order of magnitude of micromoles) under circumvention of the blood brain barrier, the dose being determined by the intensity of the d-c currents used for microelectrophoretic delivery (20 to 40 na). The topical selectivity of the deposition of these substances is assured by the dimensions of the microelectrode tip, measuring 1 to 3 μ.

This report is concerned with the effects of dexamethasone (11β, 17α, 21-trihydroxy-9 α-fluoro-16 α-methyl-pregna-1,4-diene-3,20-dione) (*9*), a synthetized corticosteriod and potent inhibitor of adrenocorticotropin release (*10*), microphoretically delivered to single neurons in rat brain. Because of its ready water solubility, the Na-dexamethasone-21-phosphate (½ molar solution) was used, after its electrophoretic mobility had been determined by paper electrophoresis at a *p*H of 7.9 (*11*). Five-barreled microelectrodes (*12*) were stereotaxically inserted into the brains of 23 male rats, weighing 260 to 350 g and anesthetized with chloralose/urethane. Action potentials were monitored visually and acoustically, stored on magnetic tape, and filmed when required (*13*). The position of the electrode tip was accurately marked by microelectrophoretic ejection of fast green (*14*) and the color spot was located histologically on 24 μ serial sections (Fig. 1C).

While Na-dexamethasone-21-phosphate applied to neurons in the cortex, hippocampus, and thalamus never influenced neuronal activity (as judged from the rate of discharge and the size or the form of the action potentials), a marked depression of the discharge rate of certain hypothalamic (Fig. 1A) and mesencephalic neurons was seen. Out of 115 neurons tested with dexamethasone in the latter areas, 15 neurons were inhibited. These cells were found in 11 animals to be concentrated in circumscribed areas of the periventricular gray of the third ventricle (Fig. 1B) (*15*) and the aqueduct; negative points were also carefully mapped. The depression of hypothalamic neuronal activity was found to be almost instantaneous and virtually complete throughout the period of drug application. The depression of mesencephalic units was somewhat more delayed and more progressive, but finally equally complete. In both cases the depression outlasted the time of microelectrophoresis by 20 to 30 seconds; thereupon the preapplication firing rate was gradually resumed.

The rapidity of this suppressive effect is reminiscent of the earlier observation (*16*) that adrenocortical steroids are capable of preventing the activation of the pituitary-adrenal axis by histamine if they are administered intravenously only 10 seconds before the application of the stressful stimulus. The delay in resuming the original rate of discharge, on the other hand, might be due to supraphysiological concentrations of the steroid achieved in the extracellular environment by this particular route of administration.

Two possible explanations for the observed suppression of single unit activity by dexamethasone microelectrophoresis are proposed: (i) these neurons are directly involved in the synthesis of CRF (corticotrophin releasing factor) and directly controlled, by negative feedback, by their adrenocortical end product, or (ii) they are independent measuring devices constantly monitoring the concentration of circulating steroids. More recent findings on the "osmoreceptors" suggest the view that these dual functions as "effectors" and "comparators" might even be combined within the same neuron (*7*). Although the exact nature of the neuronal elements depressed by dexamethasone remains at present in doubt, the technique of microelectrophoresis appears to offer an interesting new approach to neuroendocrine problems and seems to be remarkably free of certain drawbacks (for example, leakage of steroids from the site of implantation; nonspecific irritative effects of lesions) inherent in methods hitherto employed.

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References and Notes

1. M. A. Slusher and J. E. Hyde, *Am. J. Physiol.* **210**, 103 (1966); E. Endröczi and K. Lissak, *Acta Physiol. Acad. Sci. Hung.* **24**, 67 (1963).
2. J. Moll, *Z. Zellforsch. Mikroskop. Anat.* **49**, 515 (1959); A. Brodish, *Endocrinology* **73**, 727 (1963).
3. L. Martini, A. de Poli, A. Pecile, S. Saito, F. Tani, *J. Endocrinol.* **19**, 164 (1959).
4. E. Endröczi, K. Lissak, M. Tekeres, *Acta Physiol. Acad. Sci. Hung.* **18**, 291 (1961); P. G. Smelik and C. H. Sawyer, *Acta Endocrinol.* **41**, 561 (1962); J. M. Davidson and S. Feldman, *Endocrinology* **72**, 936 (1963); J. Chowers, S. Feldman, J. M. Davidson, *Am. J. Physiol.* **205**, 671 (1963); B. Bohus and E. Endröczi, *Acta Physiol. Acad. Sci. Hung.* **25**, 11 (1964); A. Corbin, G. Mangili, M. Motta, L. Martini, *Endocrinology* **76**, 811 (1965); S. Feldman, N. Conforti, J. M. Davidson, *Neuroendocrinology* **1**, 228 (1965-66).
5. J. Szentágothai, B. Flerkó, B. Mess, B. Halász, *Hypothalamic Control of the Anterior Pituitary: An Experimental-Morphological Study* (Akadémiai Kiado, Budapest, 1962), pp. 174–191; W. Ganong, in *Advances in Neuroendocrinology*, A. V. Nalbandov, Ed. (Univ. of Illinois Press, Urbana, 1963), pp. 92–149; G. Mangili, M. Motta, L. Martini, in *Neuroendocrinology*, L. Martini and W. F. Ganong, Eds. (Academic Press, New York, 1966), vol. 1, pp. 297–370.
6. M. A. Slusher, J. E. Hyde, M. Laufer, *J. Neurophysiol.* **29**, 157 (1966); S. Feldman and N. Dafny, *Israel J. Med. Sci.* **2**, 621 (1966).
7. B. A. Cross and I. A. Silver, *Brit. Med. Bull.* **22**, 254 (1966).
8. G. C. Salmoiraghi and F. A. Steiner, *J. Neurophysiol.* **26**, 581 (1963); D. R. Curtis, in *Physical Techniques in Biological Research*, W. L. Nastuk, Ed. (Academic Press, London, 1964), vol. 5, part A, pp. 144–190.
9. We are indebted to Dr. G. Anner, CIBA Ltd., Basel, for a generous supply of the drug.
10. L. Martini, M. Fochi, G. Gavazzi, A. Pecile, *Arch. Int. Pharmacodyn.* **140**, 156 (1962).
11. This investigation was carried out by Dr. G. Rentsch, F. Hoffman-La Roche Ltd., Basel.
12. The five-barreled glass pipettes for the microelectrodes were manufactured by Dr. W. Ingold, Zurich.
13. For technical details, see F. A. Steiner and K. Ruf, *Helv. Physiol. Acta* **24**, 181 (1966).
14. R. C. Thomas and V. J. Wilson, *Nature* **206**, 211 (1965).
15. D. Albe-Fessard, F. Stutinsky, S. Libouban, *Atlas Stéréotaxique du Diencéphale du Rat Blanc* (Centre National de la Recherche Scientifique, Paris, 1966).
16. W. D. Gray and P. L. Munson, *Endocrinology* **48**, 471 (1951).

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14 March 1967

Deuterium and Oxygen-18 in Natural Waters: Analyses Compared

The International Atomic Energy Agency recently compared mass-spectrometric analyses of isotope ratios of deuterium and oxygen-18 in natural water. One aim was to enable laboratories the world over to compare published results that may refer to the same scale but may in fact differ because of systematic analytical errors or unreliable standards.

Table 1 summarizes the results from 12 laboratories (*1*) before mid-February 1967. The long-term reproducibility resulted from measurements of the same sample over a period of at least a few months. Agency (IAEA) sample 1 represents ground water, sample 2 is a mixture of two surface samples collected from the Kattegat and the Baltic Sea, and sample 3 is surface water from Greenland. Sample 2 contained 35.3 percent Cl, and 0.002 percent $HgCl_2$ was added against bacterial activity. Duplicate 20-ml samples were sent to participating laboratories in sealed glass ampoules along with samples of NBS-1 and NBS-1A, obtained in plastic containers from the National Bureau of Standards. The data relating to the NBS standards are not included, since it became obvious that the plastic containers were not suitable and that variations due to evaporation and exchange were prominent. The data presented are based on comparison with working standards (previously calibrated against a primary standard such as NBS-1 and NBS-1A) or, in a few instances, on absolute measurements.

Table 1. Analyses of ^{18}O and deuterium in three samples of water; permillage deviations from SMOW (*2*) (deviation, 1σ).

Laboratory	IAEA sample 1	IAEA sample 2	IAEA sample 3	Long-term σ (‰)
		Oxygen-18		
1	−8.88 ± 0.04	+0.58 ± 0.03*	−29.36 ± 0.05	0.07
2	−10.84 ± .4	−1.51 ± .4	−30.58 ± .4	
3	−9.02 ± .11	+0.19 ± .10	−29.39 ± .14	
5	−8.71 ± .08	+ .20 ± .09	−29.33 ± .29	
6	−9.09 ± .08	+ .33 ± .08	−29.42 ± .13	
7	−8.99 ± .03	+ .49 ± .04	−29.40 ± .06	
8	−8.93 ± .02	+ .35 ± .02	−29.11 ± .03	.07
9	−9.12	+ .31*	−29.26	
11	−9.17 ± .04	+ .20 ± .04*	−29.17 ± .04	.05
12	−8.95 ± .04	+ .29 ± .01	−29.34 ± .01	
		Deuterium		
1	−61.7 ± 0.5	+2.3 ± 0.4	−220.6 ± 0.5	1.5
2	−62.5 ± 1.0	−2.5 ± 1.0*	−215.2 ± 1.0	1.0
4	−62.2 ± 1.1	+2.3 ± 1.4	−220.3 ± 1.3	
5	−61.3 ± 1.0	−1.5 ± 1.0		
6	−62.1 ± 0.9	+0.7 ± 0.7	−221.7 ± 1.1	
7	−60.6 ± .5	+3.6 ± .4	−221.8 ± 0.3	0.28
10	−62.8 ± 1.4	+2.6 ± .7	−221.7 ± 1.0	
11	−65.7 ± 0.6	+1.8 ± .6*	−235.5 ± 0.6	.6
12	−62.41 ± .12	+2.06 ± .06	−222.6 ± .15	

* Sample not distilled.

The Agency is now following the recommendation for establishment of the new standards. Furthermore, a qualified laboratory will try to establish the absolute isotopic concentrations of the new standards. As soon as they are available, samples of the new standards, together with NBS samples, will be distributed among interested laboratories for intercomparison. The Agency thanks cooperating scientists for their collaboration and permission to publish the results.

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References

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2. H. Craig, *Science* **133**, 1833 (1961).

13 March 1967