Ineffectiveness of Adrenocorticosteroids and Adrenocorticotropin in Altering Na-K Currents in Squid Giant Axon

Adrenocorticosteroids have been shown to alter central and peripheral nervous system function in man and other animals[1-4]. Withdrawal of hormonal replacement therapy from patients with adrenal cortical insufficiency (ACI) or with panhypopituitarism produces significant increases in ulnar motor conduction velocity[1,5] whereas synaptic delay in both central and peripheral nervous systems is significantly prolonged[1]. Following adrenalectomy in rat, sciatic nerve excitability is significantly increased[6] and seizure thresholds to various stimuli are significantly lowered[4,7]. Treatment of patients with ACI and panhypopituitarism with Na-K-active adrenal corticosteroids does not alter either the increased conduction velocity or the lengthened synaptic delay, but treatment with carbohydrate-active steroids (CAS) returns both functions to normal[1,5]. Treatment of adrenalectomized rats with NaCl or with various adrenocorticosteroids lowered sciatic nerve excitability[6] and also raised seizure thresholds. These observations indicated that CAS were important in neural conduction although the mechanism by which this occurred could not be specified. In an effort to evaluate one aspect of the mechanism which adrenocorticosteroids could influence nerve function we studied the effect of these hormones on the ionic currents which are responsible for the action potential of the squid giant axon.

Methods. Squit giant axons were excised, freed of supporting tissue and other nerve fibers and placed into a conventional voltage clamp system[8]. HEPES buffer[9] in K-free artificial seawater at pH 7.4 at 4 °C was used to maintain the excised axon. The transient peak and steady state currents were measured using step pulses. Estimation of the leakage current by a linear extrapolation was obtained by using hyperpolarizing pulses. K and Na currents were obtained by subtracting the estimated leakage current for each step pulse from the steady state and transient peak currents, respectively. The conventions used to represent these data are (a) all positive currents are outward currents and (b) when the membrane potential is negative the internal potential is negative with respect to the potential.

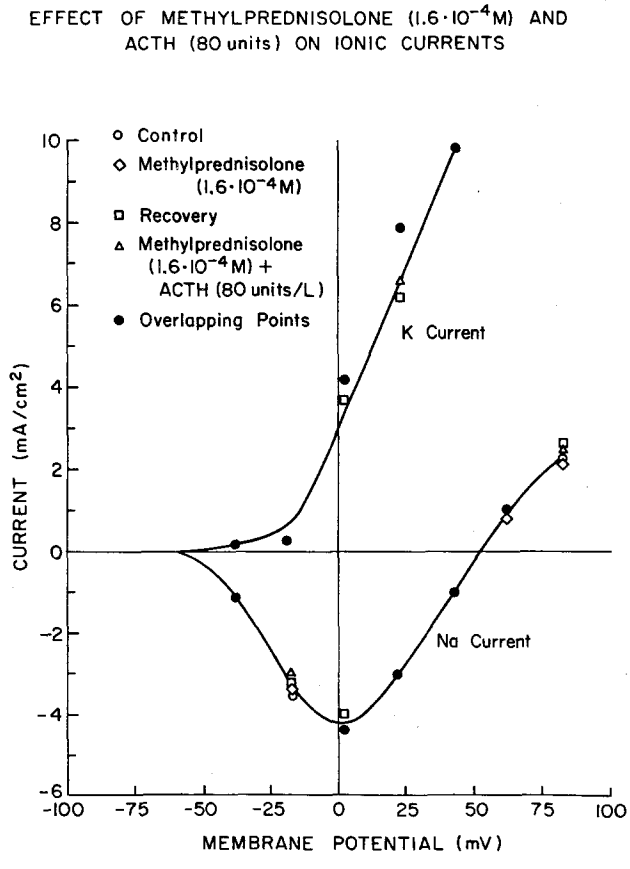

Fig. 1. Effect of methyl prednisolone, with and without ACTH on Na-K currents of squid giant axon. The currents are plotted on the ordinate, membrane potentials on the abscissa. The arrows indicate the experimental potentials at which the ionic currents were measured.

These currents were measured alone in K-free artificial seawater under control or baseline conditions and in the presence of several water soluble hormones. These included 1. 6 α-methyl prednisolone 21-sodium succinate (1.6×10^{-4} *M*), a potent carbohydrate-active steroid, used with and without adrenocorticotropin (ACTH), 80 U/l, 2. 2-methyl 9 α-fluorohydrocortisone (10^{-5} *M*), a potent Na-K active steroid and 3. ACTH alone (80 U/l). 6 α-methyl prednisolone (Solu-Medrol) and 2-methyl 9 α-fluorohydrocortisone were obtained from the Upjohn Company, Kalamazoo, Michigan as lyophyilized powders. ACTH (Acthar) was obtained from the Armour Parmaceutical Company, Chicago, Illinois also as a lyophilized powder.

Results. The effects of these hormones on the Na and K currents are shown in Figures 1 and 2. The Na-K currents are plotted on the ordinate as a function of the membrane potential which is plotted on the abscissa.

There is no difference in the measured currents upon the addition to the external solution of either the methyl prednisolone with or without ACTH (Figure 1) or 2-methyl 9 α-fluorohydrocortisone (Figure 2). The addition of ACTH alone (80 U/l) to the external solution also had no effect on the Na-K currents.

Discussion. These results demonstrate that application of large concentrations of adrenocorticosteroids or ACTH does not alter the basic ionic events which give rise to the action potential. For prednisolone the concentration used in this study was 80 times the normal circulating blood level of cortisol in man, the activity of the hormone administered being approximately 5 times the potency of circulating cortisol. For the fluorohydrocortisone, the concentration used was more than 300 times the normal circulating blood level of aldosterone in man, the administered hormone approximately 3 times the potency of circulating aldosterone.

While Na-K active steroids significantly alter the manner by which Na and K cross cell membranes[9]

[1] R. I. HENKIN, Res. Publs Ass. Res. nerv. ment. Dis. *48*, 54 (1970).
[2] W. F. CHAMBERS, S. L. FREEDMAN and C. H. SAWYER, Expl. Neurol. *8*, 548 (1963).
[3] S. FELDMAN, J. C. TODT and R. W. PORTER, Neurology *11*, 109 (1951).
[4] D. M. WOODBURY, Pharm. Rev. *10*, 275 (1958).
[5] R. I. HENKIN, J. R. GILL, J. R. WARMOLTS, A. A. CARR and F. C. BARTTER, J. clin. Invest. *42*, 941 (1963).
[6] E. B. WRIGHT and E. J. LESTER, Am. J. Physiol. *196*, 1057 (1959).
[7] V. D. DAVENPORT, Am J. Physiol. *156*, 322 (1949).
[8] J. W. MOORE and K. S. COLE, in *Physical Techniques in Biological Research* (Ed. W. L. NASTUK; Academic Press, N.Y. 1963), vol. 6, p. 263.
[9] N. E. GOOD, G. D. WINGET, W. WINTER, T. M. CONNOLLY, S. IZAWA and R. M. M. SINGH, Biochemistry *5*, 467 (1966).

2-methyl fluorohydrocortisone, a potent Na-K active steroid in high concentration, did not alter the measured currents in squid axon. Similarly, carbohydrate-active steroids significantly alter the manner by which nerve conduction takes place in man[1,5]. In patients in whom excessive endogenous secretion of carbohydrate-active steroids occur, e.g., in patients with Cushing's syndrome or with an adrenal cortical adenoma or carcinoma, peripheral nerve conduction velocity is commonly slower than normal[10]. However, a potent carbohydrate-active steroid in high concentration did not alter the measured currents in squid axon. In man and in animals in the absence of carbohydrate active steroids and in the presence of high concentrations of ACTH peripheral conduction velocity is commonly faster than normal[1,5]. However, ACTH in high concentration did not alter the measured currents in squid axon.

In order to carry out these experiments in squid axon the hormones employed had to be administered at low temperature and over relatively short time intervals. These factors could contribute to the lack of effects observed. Similarly, one may argue that the Schwann cell layer surrounding the axon could act as a barrier for these compounds and that the 10 to 30 min. time period over which they were present in the external solution might not be expected to produce any effect. However, diphenylhydantoin[11] and tetrodotoxin[12,13], substances of similar molecular weight and size to those used in the present study do alter currents in the squid giant axon within this time period. Hence, it does not appear likely that the ineffectiveness of these drugs is due to a significant Schwann cell barrier.

Since the present experiments were carried out over short time intervals any changes produced by these hormones in the Na-K pumping mechanism would not have had sufficient time for the effects to produce any change in the ionic gradients. This can be demonstrated by the results shown in both Figures 1 and 2, in that the potential at which the Na current is equal to zero is the same indicating that the Na-ionic gradient is not changed during the experiment. In other experiments, however, these hormones could possibly effect the Na-K ionic gradients thereby altering the driving force and producing subsequent changes in conduction velocity[5,6]. The present results indicate only that adrenocorticosteroids, with or without ACTH, do not alter the ionic currents responsible for the action potential.

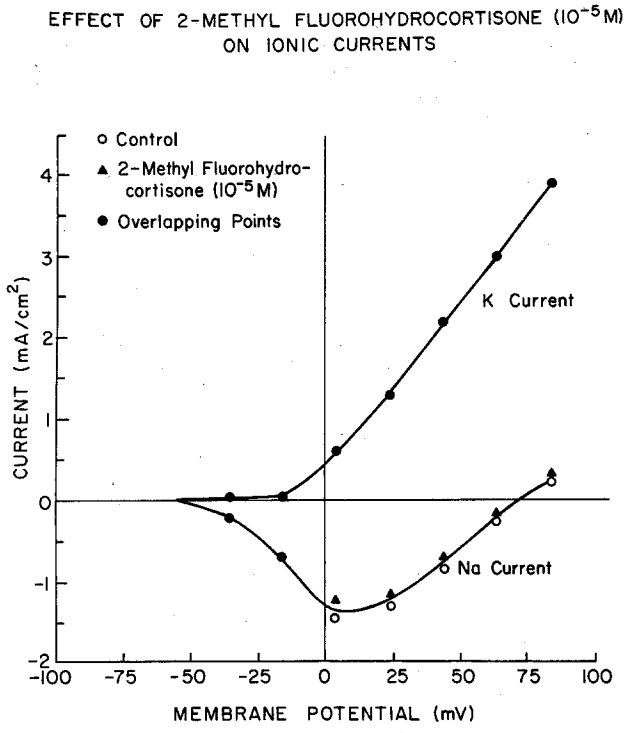

Fig. 2. Effect of 2-methyl fluorohydrocortisone on Na-K currents of squid giant axon.

Zusammenfassung. Adrenocorticosteroide und Adrenocorticotropin bewirkten in hohen Konzentrationen keine Änderungen der Na-K Ströme in Tintenfischriesenaxon; das weist darauf hin, dass die Ionenbegebenheiten, die das Axenpotential hervorrufen, durch diese Hormone nicht beeinflusst sind.

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[10] R. I. HENKIN, unpublished observations.
[11] R. J. LIPICKY, D. L. GILBERT, I. STILLMAN, Proc. natn. Acad. Sci. USA *69*, 1758 (1972).
[12] T. NARAHASHI, N. C. ANDERSON, J. W. MOORE, J. gen. Physiol. *50*, 1413 (1967).
[13] L. A. GUERVO, W. J. ADELMAN JR., J. gen. Physiol. *55*, 309 (1970).

Isolation of β_2-Microglobulin from the Urine of Patients With Itai-Itai (Ouch-Ouch) Disease

Several investigations have shown that chronic cadmium intoxication in man causes proteinuria with low molecular weight proteins[1-4]. Recently BERGGÅRD and his coworkers have isolated some low molecular weight proteins, such as β_2-microglobulin[5], free light chains[6] and retinol-binding protein[7], from the urine of patients with chronic cadmium poisoning. Moreover, the excess excretion of β_2-microglobulin[8] and free light chains[9] has been characterized in renal tubular damage with tubular proteinuria resulting from chronic cadmium poisoning.

Meanwhile, it has been considered that Itai-itai (Ouch-ouch) disease, of which main symptoms are a kind of osteomalasia and proteinuria with low molecular proteins, is caused by chronic cadmium exposure[10-12] weight. NOMIYAMA et al.[13] have suggested the presence of retinalbinding protein and β_2-microglobulin as the main