Guinea pig medial vestibular nucleus neurons in vitro respond to ACTH$_{4-10}$ at picomolar concentrations

C.L. Darlington$^{1,3}$, P.F. Smith$^{2,3}$, and J.I. Hubbard$^{1,3}$

Departments of $^1$ Physiology, $^2$ Psychology, and $^3$ the Neuroscience Research Center, University of Otago, Dunedin, New Zealand

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Summary. The responses of single guinea pig medial vestibular nucleus (MVN) neurons in vitro to adrenocorticotropic hormone, fragment 4-10 (ACTH$_{4-10}$) were recorded extracellularly. In coronal slices and in the isolated MVN, neurons were found which responded to ACTH$_{4-10}$ at picomolar concentrations (10$^{-12}$ M), indicating that ACTH$_{4-10}$ acts directly on MVN neurons and suggesting the possibility that ACTH$_{4-10}$ may act as a neurotransmitter in the MVN. In most cases where neurons responded to ACTH$_{4-10}$ (37/74 neurons), the effect was a decrease in firing. Whether or not the depressive action of ACTH$_{4-10}$ on the firing rate of MVN neurons in vitro is related to the acceleration of behavioral recovery from unilateral labyrinthectomy (vestibular compensation), which has been reported previously (Flohr and Luneburg 1982; Igarashi et al. 1985), is unclear.

Key words: Vestibular nucleus – Adrenocorticotropic hormone – Plasticity – Vestibular compensation – Guinea pig

Introduction

Neuropeptide hormones of anterior-pituitary origin have been demonstrated to affect plastic processes in both the peripheral and central nervous systems (CNS) (de Wied and Jolles 1982 for a review). Recently, short fragments of these peptide chains, without corticotropic action, such as adrenocorticotropic hormone fragment 4–10 (ACTH$_{4-10}$) and alpha-melanocyte stimulating hormone (α-MSH), have been reported to facilitate several types of lesion-induced CNS plasticity. Treatment with α-MSH has been reported to prevent the development of dopamine (DA) supersensitivity in striatal DA receptors following diencephalic hemisection in rat, facilitating the recovery from sensorimotor deficits following the hemisection (Benelli et al. 1988). Vestibular compensation, the recovery of ocular motor and postural behavior following a unilateral VIII nerve deafferentation, is facilitated by peripheral administration of ACTH$_{4-10}$ and α-MSH in frog (Flohr and Luneburg 1982; Luneburg and Flohr 1988) and by ACTH$_{4-10}$ in squirrel monkey (Igarashi et al. 1985). The mechanism by which these neuropeptides facilitate lesion-induced CNS plasticity is uncertain: they may exert their effects by acting on specific CNS nuclei which are critical for the recovery process or by having a diffuse modulatory effect on many areas of the CNS (Flohr et al. 1981).

There has been no evidence presented to suggest the probable site of action of ACTH$_{4-10}$ in accelerating vestibular compensation. ACTH-containing cell bodies or fibers have not been reported within the brainstem vestibular nuclei (Palkovits 1989 for a review). Only low concentrations of ACTH$_{1-24}$ binding sites have been reported in the brainstem and ACTH$_{4-10}$ fails to bind to the ACTH$_{1-24}$ binding sites (Hnatowich et al. 1989). At present there is no evidence for binding sites specific to ACTH$_{4-10}$.

The aim of the present study was to determine if guinea pig medial vestibular nucleus (MVN) neurons in vitro respond to ACTH$_{4-10}$, as a first step in determining whether peripherally administered ACTH$_{4-10}$ may act directly on the MVN in order to facilitate vestibular compensation (Darlington et al. 1989a, b).

Methods

Extracellular single neuron recordings were made from 74 MVN neurons in brainstem slices extracted from 21 labyrinthine-intact albino guinea pigs, male and female. The guinea pigs were anesthetized with ether and their brainstems quickly removed into chill-
ed (approximately 4°C) artificial cerebrospinal fluid (ACSF). During dissection, chilled ACSF was continuously applied to the brainstem with a pipette. Coronal slices containing the MVN (600–1000 μm thick) were cut by hand with a chilled razor blade. For 3 animals, the 2 MVN were dissected from the coronal slice so that ACTH₄⁻₁₀ could be applied to each MVN in isolation from the other MVN and adjoining nuclei (all other procedures were the same as described above). Slices were incubated in a slice chamber at 30°C for 1 h prior to recording, and superfused continuously with a standard ACSF (in mM): NaCl 126.0, KCl 5.0, KH₂PO₄ 1.25, MgSO₄ 1.3, NaHCO₃ 26.0, glucose 10.0, CaCl₂ 2.5, Phenol Red 0.05% (Darlington et al. 1989a; Smith et al. 1990). The ACSF was continuously bubbled with 95% O₂ and 5% CO₂ and maintained at a pH of 7.4. During recording the chamber temperature was maintained at 35–37°C and the ACSF flow rate set to 2 ml/min. The chamber turnover time was 2 min. The time from decapitation to immersion in the chamber was always less than 6 min.

Single neurons within the MVN were recorded extracellularly with glass micropipettes (4–10 MΩ impedance) filled with 2 M NaCl and Fast Green FCF dye, to facilitate visualization of the electrode position. The MVN could be identified during dissection when looking at the dorsal aspect of the brainstem by its proximity to the sulcus limitans and when looking at the coronal slice in the slice chamber by its proximity to the IVth ventricle. Baseline firing rate was recorded while neurons were superfused with the standard ACSF (control), then a second ACSF solution (test solution) containing the desired concentration of ACTH₄⁻₁₀ (Sigma) was turned on for 4 min, followed by a return to the control solution. If the cell stopped firing during the test solution the control solution was turned on again immediately. Four concentrations of ACTH₄⁻₁₀ were used (in M): 10⁻¹⁴, 10⁻¹², 10⁻¹¹ and 10⁻¹⁰. Preliminary experiments with concentrations higher than 10⁻¹⁰ M showed that many cells irreversibly decreased firing, suggesting that the drug may have been having neurotoxic effects. Only neurons showing reversible changes in firing rate were analysed. Firing frequency was analysed on-line using an Ortec time-histogram analyser (model 4620/4621) and histograms were plotted using an Apple IIe microcomputer. Firing rate was considered to have increased or decreased from baseline when a reversible change of greater than or equal to 20%, measured at the peak of the increase or decrease, occurred (Darlington et al. 1989a). In some experiments, each neuron was tested with 3 concentrations of ACTH₄⁻₁₀, while in other experiments only a single concentration was used.

Results

49% (31/63 neurons) of the MVN neurons in the coronal slices tested with ACTH₄⁻₁₀ responded with a significant

![Fig. 1. Percentage of MVN neurons tested at each concentration of ACTH₄⁻₁₀ showing an increase, decrease or no change in firing rate. Four concentrations are shown: 10⁻¹⁴ M (n = 18 neurons), 10⁻¹² M (n = 30), 10⁻¹¹ M (n = 20) and 10⁻¹⁰ M (n = 20), expressed as a picomolar concentration.](image)

![Fig. 2. Percentage decrease in firing rate from baseline for MVN neurons showing a decrease in firing in response to ACTH₄⁻₁₀. The solid bars indicate the mean and the hatched bars ± standard error of the mean. Four concentrations are shown: 10⁻¹⁴ M (n = 18 neurons), 10⁻¹² M (n = 30), 10⁻¹¹ M (n = 20) and 10⁻¹⁰ M (n = 20), expressed as a picomolar concentration.](image)

![Fig. 3A-C. Examples of the effect of ACTH₄⁻₁₀ on the firing rate of MVN neurons in coronal slices. Each plot represents firing rate for a single neuron, where each point represents the average number of spikes/s for a specific interval of time (bin width). A 10⁻¹² M ACTH₄⁻₁₀, bin width 5 s. B 10⁻¹⁴ M ACTH₄⁻₁₀, bin width 10 s. C 10⁻¹⁴ M ACTH₄⁻₁₀, bin width 5 s. “ACTH” indicates the onset of the ACTH₄⁻₁₀ solution; “C” indicates the onset of the control solution.](image)