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BRAIN RESEARCH

Research Report

Protein kinases regulate glycine receptor binding in brain stem auditory nuclei after unilateral cochlear ablation

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ABSTRACT

Glycinergic synaptic inhibition is part of acoustic information processing in brain stem auditory pathways and contributes to the regulation of neuronal excitation. We found previously that unilateral cochlear ablation (UCA) in young adult guinea pigs decreased [^{3}H] strychnine binding activity in several brain stem auditory nuclei. This study determined if the UCA-induced deficit could be regulated by protein kinase C (PKC), protein kinase A (PKA) or Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). The specific binding of [^{3}H] strychnine was measured in slices of the dorsal (DCN), posteroventral (PVCN) and anteroventral (AVCN) cochlear nucleus (CN), the lateral (LSO) and medial (MSO) superior olive, and the inferior colliculus (IC) 145 days after UCA. Tissues from age-matched unlesioned animals served as controls. UCA induced deficits in specific binding in the AVCN, PVCN, and LSO on the ablated side and in the MSO bilaterally. These deficits were reversed by 3 μM phorbol 1,2-dibutyrate, a PKC activator, or 0.2 mM dibutyryl-cAMP, a PKA activator. However, 50 nM Ro31-8220, a PKC inhibitor, and 2 μM H-89, a PKA inhibitor, had no effect in unlesioned controls and after UCA. In contrast, 4 μM KN-93, a CaMKII inhibitor, relieved or reversed the UCA-induced binding deficits and elevated binding in the IC. These findings suggest that a UCA-induced down-regulation of glycine receptor synthesis may have occurred via reduced phosphorylation of proteins that control receptor synthesis; this effect was reversed by diminishing CaMKII activity or increasing PKC and PKA activity.

1. Introduction

Glycinergic synaptic inhibition in brain stem auditory pathways is an important component of acoustic information processing and, together with GABAergic inhibition, contributes to the regulation of neuronal excitation (Caspary et al., 1994; Faingold et al., 1991; Grothe and Sanes, 1994; Backoff et al., 1999; Davis and Young, 2000). Glycinergic inhibition, however, may become impaired after sensorineural hearing loss, which usually involves damage to the cochlea and degeneration of the cochlear nerve. Destruction of the cochlear nerve in young adult guinea pigs reduces [^{3}H] strychnine binding in several auditory brain stem nuclei on the ablated side without affecting glycine transmitter release

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Abbreviations: ANOVA, analyses of variance; AVCN, anteroventral cochlear nucleus; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; CN, cochlear nucleus; DBcAMP, dibutyryl-cAMP; DCN, dorsal cochlear nucleus; DMSO, dimethyl sulfoxide; GABA, gamma-amino buytric acid; IC, inferior colliculus; ICc, central nucleus of the inferior colliculus; i.p., intraperitoneal; LSO, lateral superior olive; MSO, medial superior olive; PBS, phosphate buffer saline; PDBU, phorbol 1,2-dibutyrate; PKA, protein kinase A; PKC, protein kinase C; PVCN, posteroventral cochlear nucleus; s.c., subcutaneous; UCA, unilateral cochlear ablation

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(Suneja et al., 1998a,b; Potashner et al., 2000). This implies a decline in glycine receptor activity, which may contribute to additional pathological symptoms that often accompany sensorineural hearing loss, such as tinnitus, loudness misperceptions, and poor isolation of important sounds in a background of noise (Olson et al., 1975; Jastreboff, 1990; Salvi et al., 2000). Several protein kinases are associated with postsynaptic sites (Liu and Jones, 1996; Ramakers et al., 1997; Colbran, 2004) and phosphorylation of the glycine receptor alters its properties (Smart, 1997; Gentet and Clements, 2002). It is conceivable, therefore, that the actions of protein kinases might be involved in the change of glycinergic receptor activity after UCA. To assess this possibility, we quantified the specific binding of [^{3}H]strychnine, using microdissected slices of brain stem auditory nuclei 145 days after UCA, when the deficit in glycine receptor activity is fully developed (Suneja et al., 1998a; Potashner et al., 2000). We compared this binding activity to that in tissues of age-matched, unlesioned control animals. A proportion of the tissues from both the controls and the ablated animals were treated with activators or inhibitors of PKC, PKA or CaMKII to determine their effects on [^{3}H] strychnine binding. Some of the findings have been reported in an abstract (Yan et al., 2004).

2. Results

The auditory nuclei from unlesioned controls exhibited two levels of binding activity. The CN subdivisions and the LSO bound 0.12–0.18 fmol of strychnine per mg of tissue protein,

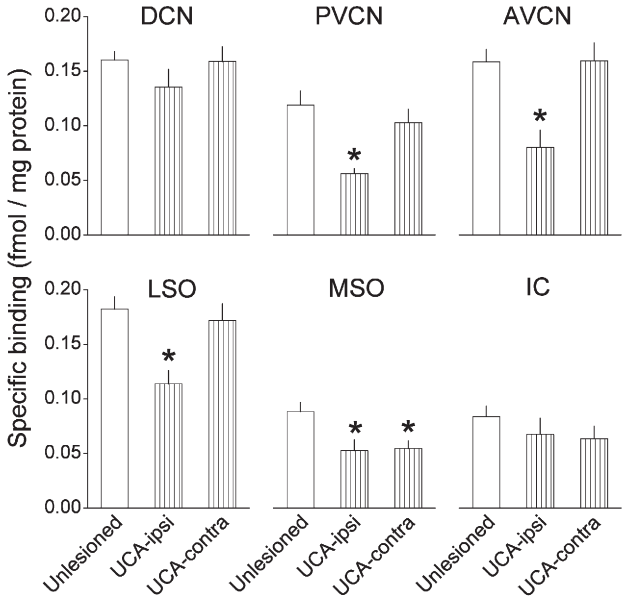

Fig. 1 – Effects of UCA on specific binding of [^{3}H]strychnine in several of the brain stem auditory nuclei. [^{3}H]strychnine binding was measured 145 days after UCA, as described in Experimental procedures, where the number of animals in each group is provided. Asterisks denote significant differences from unlesioned controls (Duncan's multiple comparison test; $P \leq 0.05$).

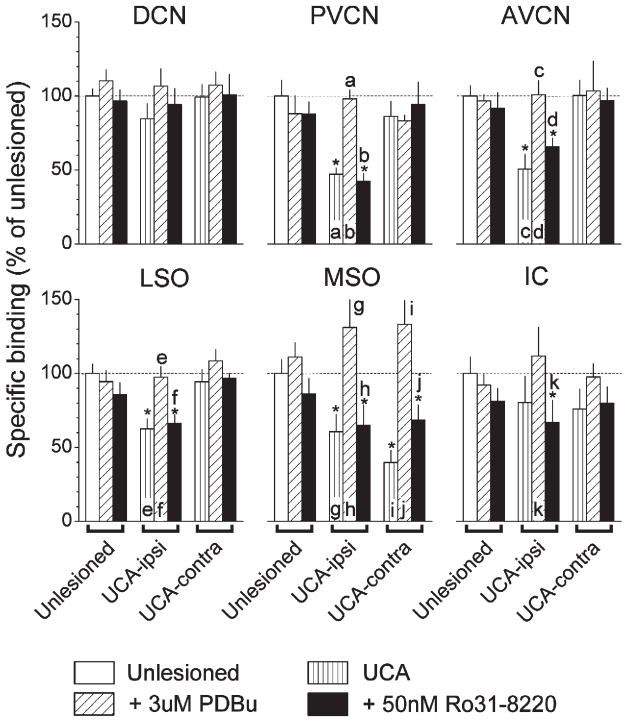

Fig. 2 – Actions of PDBu, a PKC activator, and Ro31-8220, a PKC inhibitor, on the specific binding of [^{3}H]strychnine. Values from Fig. 1 for binding in unlesioned controls and animals that received the UCA are reproduced here for convenience. PDBu elevated binding that was deficient after UCA but had little or no effect where binding was normal. Ro31-8220 did not alter binding. Asterisks denote significant differences from unlesioned controls (Duncan; $P \leq 0.05$). A lower case letter above a bar denotes a significant difference from the bar containing the same letter (Duncan; $P \leq 0.05$).

while the MSO and IC bound approximately 0.08 fmol per mg protein (Fig. 1, white bars).The relative levels of binding activity determined in the present 400-μm-thick samples of the auditory nuclei, quantified with liquid scintillation spectrometry, were similar to those observed in 15-μm-thick samples, quantified autoradiographically (Suneja et al., 1998a). The autoradiographic study indicated that the distribution of binding activity was similar to that of high glycine concentrations, suggesting a corresponding distribution of receptors and glycine presumed to be in presynaptic endings.

One hundred and forty-five days after UCA, binding declined in the PVCN, AVCN, and LSO on the ablated side by 53%, 49%, and 37%, respectively (Fig. 1, vertically hatched bars). Binding also declined bilaterally in the MSO by 40%. Apparent declines of 15–24% in the DCN and IC did not achieve statistical significance. The magnitudes of these postablation deficits resembled those observed in the autoradiographic material.

Treatment with activators or inhibitors of PKC (Fig. 2), PKA (Fig. 3), and CaMKII (Fig. 4) did not alter strychnine binding in tissues taken from unlesioned controls (see Unlesioned, compare white bars to diagonally hatched and black bars). By contrast, in tissues taken from UCA animals, both the PKC activator, PDBu (Fig. 2), and the PKA activator, DBcAMP (Fig. 3),