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Dynorphin release by the lateral olivocochlear efferents may inhibit auditory nerve activity: A cochlear drug delivery study

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HIGHLIGHTS

- The kappa opioid receptor agonist (−)pentazocine was applied to the cochlea.
- Sound evoked neural potentials were reduced in amplitude after drug treatment.
- Endogenous dynorphin at biologically plausible levels has an inhibitory effect.
- Higher (nonbiological) concentrations of drugs excite the auditory nerve.
- Transmitter effects should be assessed at biologically plausible concentrations.

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ABSTRACT

Dynorphin (dyn) is suggested to excite the auditory nerve (AN) when released by the lateral olivocochlear (LOC) efferents. However, previous studies evaluated either intravenously delivered dyn-like agents, raising the potential for systemic (central) effects, or agent concentrations unlikely to be achieved via endogenous cochlear release. This study tested the hypothesis that biologically relevant increases in dyn levels in the cochlea achieved via diffusion of the drug of (−)pentazocine across the round window membrane enhances AN firing. In general, amplitude of the cochlear whole-nerve action potential (CAP) was depressed following drug application. These results suggest that dyn released by the LOC neurons would likely act as an inhibitory transmitter substance in the LOC system; neurotransmission is one of the LOC system's vast unknowns.

1. Introduction

Dynorphin (dyn) was suggested as a cochlear transmitter after dyn-B detection in homogenized cochlear tissue [15]. Dyn-like immunoreactivity is localized to the lateral superior olive (LSO), origin of lateral olivocochlear (LOC) efferents innervating the cochlea [1,3], and LOC terminations on auditory nerve (AN) dendrites [2]. Some LSO cell bodies co-localize enkephalin (enk) and dyn [1,3] or dyn and choline acetyltransferase [ChAT: enzyme responsible for synthesizing acetylcholine (ACh)] [1]. Dyn-B and α-neoendorphin immunostaining are found under the cochlear inner hair cells (IHCs), and in tunnel spiral bundle [TSB, containing LOC neurons traveling from LSO to cochlea] and inner spiral bundle [ISB, composed of both LOC neurons at bases of IHCs and medial (MOC) olivocochlear neurons under outer hair cells (OHCs)] [2,42–44]. Dyn opioid peptides primarily act as agonists at kappa-opioid receptors (KOR) but also have some affinity for μ-opioid receptors (MOR), which they antagonize. KOR and MOR immunoreactivity have been confirmed in rat [17] and guinea pig [16] cochleae. Unlike the MOC system, very little is known about the LOC system, including LOC neurotransmission.

Dyn-like substances delivered intravenously have robust effects on sound-evoked neural potentials. Lower (better) sound-evoked compound-action-potential (CAP) thresholds and enhanced (larger) CAP amplitude were observed for stimuli at 0–20 dB sensation level (SL) after 8–16 mg/kg (−)pentazocine or 20 mg/kg U50488 delivered intravenously (but not 6 mg/kg U50488) [34,37]. Excitation was attributed to KOR binding in the cochlea as selective KOR antagonists naloxone (applied intravenously, see [35]) and norbinaltorphimine (norBNI) (applied to the round window membrane (RWM), see [38]) blocked these effects.

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When 50-mM U50488 was applied to the RWM to eliminate potential systemic effects, CAP amplitude increased at 0–10 dB SL but decreased at 20–40 dB SL [39]. "Biphasic" effects were replicated using 50-mM (−)pentazocine and 100-mM U50488 [32]; however, high drug concentrations may induce non-selective effects. Endogenous cochlear opioid release is suggested to achieve *nanomolar*, or perhaps *micromolar*, concentrations [28]. Sharply contrasting with this, 50-mM (−)U50488 applied to chinchilla RWM might produce 1.1 mM cochlear drug concentrations [Washington University Cochlear Fluids Simulator (version 1.6i, December 2005; http://oto.wustl.edu/cochlea/)]. The current study assessed whether micromolar cochlear dosing of (−)pentazocine mediates AN activity.

2. Methods

2.1. Subjects

Male and female pigmented guinea pigs (Elm Hill Breeding Labs, Chelmsford, MA) were maintained with free access to food (Guinea Pig Chow, PMI Nutrition International, Inc., Brentwood, MO) and water ($n = 11$). Animal weights were ~250–300 g on arrival and ~350–500 g at study entry. The animal care program was AALAC accredited. Husbandry met or exceeded all applicable standards; the University (of Michigan) Committee on Use and Care of Animals approved all protocols.

2.2. Surgical procedure

Guinea pigs were anesthetized (108 mg/kg ketamine, 14 mg/kg xylazine) and the cochlea exposed via post-auricular incision. A platinum–iridium-wire ball-electrode (diameter = 0.2–0.25 mm) was placed through the wall of the cochlea into scala tympani. A silastic bead 0.5-mm distal to the end of the electrode prevented over-insertion and cochlear fluid loss (as in [23]). The electrode was secured temporarily using cyanoacrylate (VetBond) at the bulla, then cemented securely to the bulla (Durelon). CAP was assessed immediately after securing the electrode ("Baseline"), and 30-min after application of artificial perilymph (AP) (145-mM NaCl, 2.7-mM KCl, 2.0-mM $MgSO_4$, 1.2-mM $CaCl_2$, 5.0-mM HEPES; pH = 7.40, osmolality = 280–285 mOsm) or increasing concentrations (1.0, 5.0 mM) of (−)pentazocine (Sigma #P-134, CAS 66429-56-9) to the RWM. (−)Pentazocine was initially dissolved in a small volume of dilute (0.1 M) hydrochloric acid then brought to the appropriate concentration using AP (final pH = 6.73–7.08). RWM applications were ~6-μl, and the middle ear was carefully dried prior to CAP tests using fine tip cotton points.

2.3. CAP

Acoustic stimuli were tone-pips from 0 to 100-dB SPL (5-dB increments, 5-ms duration, 0.5-ms rise-fall; 10/s). Control subjects receiving AP were tested from 2 to 18 kHz in 2-kHz increments (nominally 9-min); (−)pentazocine subjects were tested at 2, 4, 8, and 16 kHz (nominally 4-min). Signals were generated using Tucker-Davis Technology (TDT; Alachua, FL) hardware and SigGen32 software. Signals were converted to analog (DA1), filtered (FT6-2, $F_c = 40$ kHz), attenuated (PA5), and presented using a 200-Ohm transducer (Beyer Dynamic, Farmingdale, NY) coupled to the ear canal via vinyl tubing. Cochlear potentials were filtered (300–3000 Hz) and amplified (1000×) using a Grass P55 amplifier. BioSig32 was used to average 25 evoked responses per frequency/level combination. CAP threshold was defined as the level producing a 10-μV response using linear interpolation. All subjects were required to be within 2-SD of the mean for the population of animals tested in this laboratory previously, for both threshold and amplitude, at baseline.

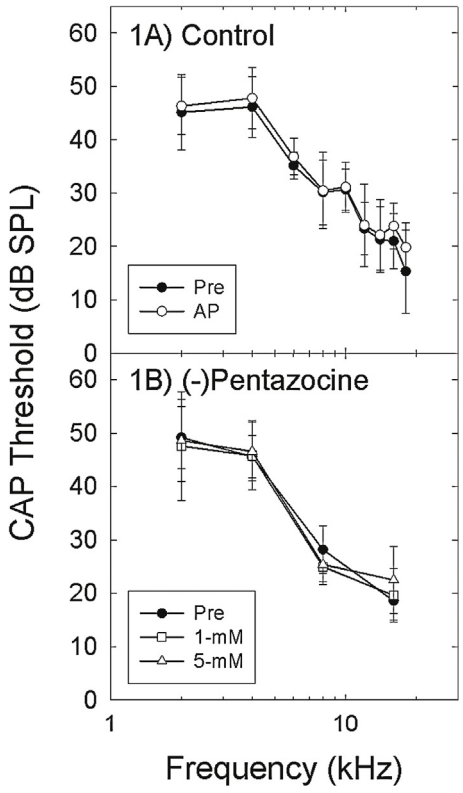

Fig. 1. There were no statistically reliable changes in CAP threshold (mean ± S.D.) after artificial perilymph (AP) control (1A, $n = 6$), or (−)pentazocine (1B, $n = 5$).

2.4. Statistical analysis

All values are mean ± S.D.; statistical reliability of group differences was analyzed via ANOVA using SPSS 13.0. Statistical reliability of drug effects was evaluated at each frequency using mixed-effects ANOVA for the between-subjects condition (baseline, 1-mM, and 5-mM) and within-subjects effect of stimulus level (0, 5, 10,. . ., and 100-dB SPL). Greenhouse–Geisser corrections adjusted for sphericity violations; Bonferroni corrections were applied for multiple comparisons.

3. Results

3.1. CAP threshold

Threshold sensitivity was not reliably affected by AP (Fig. 1A) or 1- or 5-mM (−)pentazocine (Fig. 1B). There was no statistically reliable difference between Baseline versus AP ($F = 1.008$, df = 1, 7, $p = 0.349$) or Baseline vs 1- or 5-mM (−)pentazocine ($F = 1.813$, df = 1.775, 7.098, $p = 0.230$).

3.2. CAP amplitude (dB SPL)

Both 1- and 5-mM (−)pentazocine depressed CAP amplitude (Fig. 2). All pair-wise comparisons between drugs and baseline were statistically significant after Bonferroni corrections, except Baseline vs 1-mM (−)pentazocine at 2-kHz. Drug effects were most robust at higher frequencies (expected with RWM delivery, see [6]) and higher sound levels [Treatment × Level interaction significant for pre vs 5-mM at 8- and 16-kHz (all p's < 0.05)]. In contrast to (−)pentazocine, AP did not affect CAP amplitude (all p's > 0.05).