INTRODUCTION

The primary neurotransmitter released by the efferent terminals of the olivocochlear pathway is acetylcholine (ACh), which binds to nicotinic $\alpha_9$-$\alpha_{10}$ located at the base of the outer hair cell (OHC) (Taranda et al., 2009), and M2 and M4 muscarinic receptors, in the synaptic complex of both the lateral olivocochlear pathway (LOC) and the medial olivocochlear pathway (MOC) (Maison et al., 2010). ACh and the calcitonin gene-related peptide co-localize in the efferent neurons of the OC bundle. Immunoreactivity to $\gamma$-aminobutyric acid was also detected in the OC cochlear efferents, although it seems to be limited to the apex of the cochlea (Maison, Adams, & Liberman, 2003; Maison, Casanova, Received: 3 June 2019 | Revised: 7 August 2019 | Accepted: 8 August 2019
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RESEARCH ARTICLE

Opioid modulation of cochlear auditory responses in the rat inner ear

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Abstract
The auditory system has an extensive efferent innervation, which contributes to processes of control and regulation of the afferent input. The expression of receptors to various neurotransmitters and neuropeptides in the inner ear has been described, among which endogenous opioid receptors are found. The role of opioid receptors in the cochlea is not yet fully defined, it has been reported that opioid agonists and antagonists modulate the response to auditory stimuli and in clinical practice, multiple cases have been reported in which the consumption of opioid derivatives induce sensorineural hearing loss. In this work, we evaluated the effects of acute treatment with morphine, fentanyl, tramadol, and naloxone, in the auditory brain stem potentials (ABR), the compound action potential (CAP), and distortion products otoacoustic emissions (DPOAE), across a wide range of stimulus frequencies and amplitudes. Adult Long-Evans rats of the strain CII/ZV weighing 180–220 g were used. For the ABR recording drugs were administered intraperitoneally or intravenously. For the CAP and DPOAE drugs were applied by direct perfusion in the middle ear. The opioid agonists produced a consistent increase in the amplitude of the P1 component of the ABR and of the N1-P1 amplitude of the CAP. Naloxone produced no significant changes in the ABR and a reduction of the CAP N1-P1 amplitude. Also, opioid agonists induced a decrease in the amplitude of the DPOAE. These results show that the opioid receptor activation modulates both the afferent response at both the afferent response to acoustic stimuli, and also at the cochlear mechanics as revealed by DPOAE changes. These results present a significant step in understanding how opioid modulation of auditory responses may contribute to the auditory processing and to sensorineural hearing loss produced by opioids.

KEYWORDS
auditory loss, drug abuse, fentanyl, morphine, tramadol

1 | INTRODUCTION

The primary neurotransmitter released by the efferent terminals of the olivocochlear pathway is acetylcholine (ACh), which binds to nicotinic ACh $\alpha_9$-$\alpha_{10}$ located at the base of the outer hair cell (OHC) (Taranda et al., 2009), and M2 and M4 muscarinic receptors, in the synaptic complex of both the lateral olivocochlear pathway (LOC) and the medial olivocochlear pathway (MOC) (Maison et al., 2010). ACh and the calcitonin gene-related peptide co-localize in the efferent neurons of the OC bundle. Immunoreactivity to $\gamma$-aminobutyric acid was also detected in the OC cochlear efferents, although it seems to be limited to the apex of the cochlea (Maison, Adams, & Liberman, 2003; Maison, Casanova,
Holstein, Bettler, & Liberman, 2008). Dopamine and serotonin were also found in neurons innervating type I afferents, serotonin also innervates OHC (Maison et al., 2012). Enkephalins and peptides related to the family of endogenous opioids have also been detected in the LOC system (Guinan, 1996; Fex & Altschuler, 1981; Pujol, Lavigne-Revillard, & Lenoir, 1998).

Various works demonstrated that enkephalins and dynorphins are present in the LOC system and that other opioid peptides are also present in the efferent neurons of the LOC and MOC systems, probably co-expressed with ACh. The removal of the efferent innervation of the cochlea associated with a loss of enkephalergic immunoreactivity (Eybalin, 1993). Enkephalins were found to inhibit the adenyl cyclase activity in the guinea pig cochlea, and the enkephalin content of the cochlea was altered by noise stimulation (Drescher, Drescher, & Medina, 1983; Eybalin, Pujol, & Bockaert, 1987). Immunoreactivity to met- and leu-enkephalin-like was observed in the efferent terminals of the human OHC and IHC region (Scholtz, Kanonier, & Schrott-Fischer, 1998) and, in situ hybridization shown that cells expressing mRNA coding for proenkephalin were localized in rat pontine neurons, specifically in the ventral nucleus of the trapezoid body (Hoffman et al., 1993). Functional studies in the chinchilla shown that the Kappa opioid receptor (KOR) activation produced an increase in N1 and N2 components of the auditory brainstem responses, suggesting an opioid role on the LOC efferent innervation (Sahley, Kalish, Musiek, & Hoffman, 1991). In baboons, buprenorphine and morphine decrease auditory discrimination, in addition to slowing the response to sound stimuli (Hienz, Zarcone, & Brady, 2001).

The mRNA for Mu opioid receptor (MOR), Delta opioid receptor (DOR), and KOR were detected in rat and guinea pig cochlea by RT-PCR (Jongkamonwiwat et al., 2003, 2006). The MOR, KOR, and DOR immunoreactivity was detected in the IHC and OHC, and the inner- and outer-spiral bundle (ISB and OSB, respectively) underneath the base of hair cells where they co-localized with synaptophysin. The spiral ganglion somas were also intensely stained by the three receptor antibodies. Synaptophysin and β-endorphin colocalized around the spiral ganglion neurons and with MOR in the ISB and OSB. The DOR immunoreactivity was co-localized with synaptophysin and with leu-enkephalin at the ISB, OSB (Jongkamonwiwat et al., 2006). The expression of DOR mRNA levels steadily increased from P0 to P8 with no further increases by P16. KOR mRNA was expressed at a high level at P0 and P4 followed by a decrease while MOR mRNA was expressed at a low level at P0 and P4 followed by an increase by P8 and at P16 DOR staining increased steadily with development from P0 to P16 (Phansuwan-Pujito et al., 2003).

In the human spiral ganglion (SG) neurons immunoreactivity to MOR was identified in most of the neurons. A similar pattern of labeling was found with in situ hybridization. In the mouse SG neurons also most of the neurons were immunoreactive to MOR. In the organ of Corti, MOR was expressed in IHC and OHC, and the fibers underneath the IHC were also labeled (Nguyen, Mowlds et al., 2014).

In clinics, it was found that a significant number of reports have shown that auditory loss may be associated with opiate drugs. Problem is significant because over 650,000 opioid prescriptions are dispensed in the United States every day (Rawool, 2016). In this work, we evaluated the effects of acute treatment with morphine, fentanyl, tramadol, and naloxone, using auditory brainstem response (ABR), otoacoustic emissions by distortion products (DPOAE) and compound action potential (CAP) records in rats.

2 | MATERIAL AND METHODS

2.1 | Animals

The study was performed in strict accordance with the recommendations in the Guiding Principles in the Care and Use of Vertebrate Animals in Research and Training of the American Physiological Society and with the regulations of the General Health Law Research Subject Health of the Ministry of health of México. The protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the Autonomous University of Puebla (VIEP-BUAP). All efforts were made to minimize animal suffering and to reduce the number of animals used. Long Evans rats of the strain CII/ZV rats weighing 180–220 g were used. The animals were provided by the ‘Claude Bernard’ animal facility of the Autonomous University of Puebla.

The animals were anesthetized with intramuscular ketamine-xylazine 63 mg/kg and 14 mg/kg, respectively, additionally we administered atropine at a dose of 0.05 mg/kg subcutaneously and the absence of a palpebral reflex and no withdrawal of the legs induced by nociceptive stimulus (puncture in the bearing of the hind leg), were taken as parameters of an adequate anesthesia. The temperature was recorded with a digital BD thermometer and was maintained in the range of 36.5–37.5°C, with a heating system for small species developed in the laboratory. In the anesthetized rat, an otoscopic examination was performed to evaluate the tympanic membrane. Only rats with a translucent eardrum without vascularization and free of impacted earwax were included in the study.

2.2 | Auditory brainstem responses

For these experiments, tracheal intubation and insertion of an intravenous catheter in the external jugular were performed. The rats were ventilated with a respirator for small species (model 683, Harvard Apparatus); 2 ml of air are administered, at a rate of 60 breaths/minute. After the tracheotomy, the external jugular vein was exposed to insert the intravenous catheter, and the combination of ketamine-xylazine anesthesia was administered intravenously with an average perfusion rate of 7 μl/min, using an infusion pump (model 44 from Harvard Apparatus). The nociceptive response and the rectal temperature were monitored every 10 min.
The design of the acoustic stimulus, as well as the recording and analysis of the bioelectric activity, was performed using the RZ6 Sys3 multiprocessor from Tucker-Davis Technologies (TDT). The sound stimuli were produced by magnetic loudspeakers model MF1 (TDT), in order to ensure the amplitude of the acoustic stimulus was at the correct level, we constantly made the calibration of the speaker output using a Piezotronics PCB calibration device. For the calibration sweeps of various frequencies were passed and the maximum level calibrated in the closed field was 80 dB SPL. The calibration data were stored and used in each experiment. Three subcutaneous electrodes, an active electrode in the vertex, a reference electrode behind the right ear and a ground electrode behind the left ear were inserted for the recording. The recordings were made in the closed field configuration and the auditory response generated by 300–500 monaural acoustic stimuli were averaged (Shaw, 1988). The acoustic stimulus was either a click or a tone pip at frequencies of 2, 4, 8, 16, 22, 32, and 48 kHz, the duration of the tone was 5 ms, with a rise/fall phase of 0.5 ms, and presented at a repetition rate of 21/s; the level of the stimulus was decremented in intervals of 5 dB SPL in a range of 80–10 dB SPL, in order to find the auditory threshold in each of the test frequencies. The temporal stability of the auditory threshold was evaluated as well as the amplitude of the first component in each sound level of the test. The recorded electrophysiological response was preamplified in the RA4LI (TDT) head at 20X, filtered in a bandwidth of 0.1–3 kHz and visualized in the BioSigRZ program; the sampling rate of the record was 25 kHz. The recordings of the auditory responses were stored for further analysis, which consisted in evaluating the auditory threshold, amplitude, and latency of the components of the auditory potentials measured at 60 dB SPL, in the control condition, and after drug administration.

To evaluate the stability of the CAP response, saline solution was administered and the CAP response was recorded at 2, 20, and 40 min after perfusion (n = 6). It was found that the threshold, the amplitude, and latency for PI, PII, PIV or later components during 2 hr did not show significant changes. Likewise, we evaluated the ABR response with tone pips of 1.5 and 10 kHz during 90 min (n = 3), the amplitude and latency of PI, PII, and PIV components and the ABR threshold remained without significant variation during this period.

### 2.3 | Record of the CAP of the auditory nerve

In the anesthetized animal, a reference electrode was inserted in the posterior region of the neck and a ground electrode in the hind paw ipsilateral to the ear that was recorded. Then, the head of the rat was fixed to make a lateral cut of the posterior region of the animal ear toward the midline. The auditory bulla was exposed by blunt dissection in order to access to the middle ear cavity and locate the registration site in the round window. The CAP recording was made with silver wire electrode content in a glass capillary. For perfusion the BAS microperfusion system, model MD-1001 was used, the perfusion was carried at a rate of 10 μl/min for 30 min, repeated measurements were taken at 2, 20, and 40 min after perfusion of the drug. The CAP was triggered by a click stimulus of 0.1 ms and a decreasing amplitude of 70 to 10 dB SPL, presented at a repetition rate of 21/s. The stimulation was done in closed field and 256 responses were averaged for each sound level, the latency was measured at N1, the amplitude of the CAP was defined as the value of N1–P1 at 60 dB SPL, and the auditory threshold was defined as the level minimum sound where the component N1-P1 of the action potential is appreciated.

To evaluate the stability of the CAP response, saline solution was administered and the CAP response was recorded at 2, 20, and 40 min after saline perfusion (n = 6). The threshold, the amplitude of N1-P1 and the latency of N1 did not show significant changes.

### 2.4 | Distortion products otoacoustic emissions

For the recording of the DPOAE we use a low noise microphone ER-10B+ (Etymotic Research). For the auditory stimulation, magnetic loudspeakers model MF1 (TDT) was used. The MF1 speaker output was coupled to the probe containing the ER-10B+ microphone, and the entire assembly was placed in the rat auditory canal, near the tympanic membrane, to record the otoacoustic emissions caused by distortion products resulting from auditory stimulation at the frequencies tested. The acoustic stimulus consisted of two tones (f1 and f2) centered on the frequencies of 2, 4, 8, and 16 kHz. The f2/f1 ratio was 1.2 and the amplitude of f1 (L1) was 10 dB SPL greater than the amplitude of f2 (L2), the sound levels were decreasing from 80 to 10 dB SPL in intervals of 5 dB SPL, the rate of presentation of the stimulus was 47.7/s. A total of 25 responses were averaged and the frequency spectrum was recorded, which allowed us to identify the distortion products (DP) (2f1-f2) originated in the cochlea. Subsequently, the amplitude of the DP was measured at each sound level of stimulation defining the threshold of appearance of the DP based on two criteria: (a) that the trace should exceed 6 dB SPL above two standard deviations from the average of the background noise considering three bins of frequency above and below the DP, (b) the trace had to have a good index of minimum signal-to-noise ratio of 3 dB SPL; based on these criteria, the DPOAE threshold is reported.

### 2.5 | Drugs

Drugs were dissolved in saline solution before experiments. Xilazine clorhidrate, ketamine clorhidrate, morphine, and fentanyl were all of them form Pisa (Pisa Farmaceutica, Mexico City). Tramadol was from Formenti (Milan, Italy), naloxone was purchased from Sigma Chemicals (St. Louis, MO). They were kept in aliquots under fabricant specified conditions.
2.6 | Data analysis

For the statistical analysis of the temporal course of the drug’s effects on ABRs and CAP, we used a repeated-measures analysis of variance (ANOVA) follow up with post hoc Fisher’s Least Significant Difference test. For the temporal course of the drug’s effects on the DPOAEs we used repeated measures ANOVA followed by post hoc Holm–Šidák test. In the case of ABRs without a temporal course, we used a paired Student’s t test. A value of $p \leq .05$ was considered significant.

3 | RESULTS

3.1 | Morphine effects in ABR, CAP, and DPOAE

The effect of morphine was evaluated in the amplitude, latency, and threshold of the components of the ABR in response to the click type acoustic stimulus (80 dBa). The morphine was administered 6 mg/kg via i.p. and ABR recorded through 2 hr ($n = 4$). We found an increase in the amplitude of PI ($p = .03$), and an increase in the amplitude of PII, 60 min after administering morphine ($p = .001$), without changes in the amplitude of PIV ($p > .05$). The latencies of PI, PII, or PIV were not modified ($p > .05$). The effects of morphine were also evaluated in the ABR components evoked by tone pips at three frequencies (1, 5, and 10 kHz, 57 dB, $n = 3$). In this group of experiments, an increase in the amplitude of the PII component was found in all the frequencies tested ($p > .05, n = 3$), with no consistent effect in the amplitude of PI ($p > .05, n = 3$). The latencies were not consistently modified, even after 90 min after the administration of morphine. Thence, we studied the effect of morphine (132 mM) applied locally on the middle ear in the CAP recordings ($n = 8$). A tendency to increase the amplitude of the response was found, however, this change was not significant even after 40 min of morphine administration, likewise, the latency of the CAP and threshold showed no modification (Figure 1). To further analyze the effects of morphine in the cochlear activity, its effect was studied in the DPOAE recordings ($n = 6$) produced by a 60 dB SPL acoustic stimuli centered in different frequencies (indicated); morphine [132 mM] produced a non-significant increase in the threshold of DPOAE with $f_2 = 4.4$ kHz (from 55 ± 2.2 to 59.2 ± 2.4, $p = .24, n = 6$) (Figure 2a,b). The amplitude of the DPOAEs decreased in all cases at 20 and 40 min with $f_2 = 4.4, 8.7, and 17.4$ kHz (Figure 2c,d).

3.2 | Fentanyl effects in ABR

Fentanyl is a potent unspecific opioid receptor agonist, in our experiments was administered i.p. (50 μg/kg). In the ABR recordings, fentanyl produced a consistent increase in the amplitude of the PI component of the ABR produced at 5 and 10 kHz when stimulated with an intensity of 57 dBa ($p = .017$ and $p = .038$, respectively, $n = 10$) (Figure 3). The latency of the PI component showed a significant decrease in the 5 kHz tone ($p = .016, n = 10$). For the PII or PIV components, there were no changes in amplitude or latency.

3.3 | Tramadol effects in ABR, CAP

Tramadol actions were studied using ABR and CAP recordings. For the ABR recordings, the dose was 10 mg/kg i.v. ($n = 8$) Different cochlear regions were evaluated using seven different tones (2, 4, 8, 16, 22, 32, and 48 kHz). The intensity of 60 dB SPL was measured and the hearing threshold, amplitude and latency of PI for each tone were evaluated. A slight decrease in the amplitude of the PI component was found when...
stimulating with 2,000 Hz (p = .01, n = 8, paired Student t test), without significant changes in latency. For the rest of the tones, there are no changes in amplitude or latency. It was also found that the threshold increased significantly in the response to 48 kHz (p = .05, n = 6, repeated measures ANOVA), without changes in the threshold for the other tones (Figure 4).

For the study of the effect of tramadol in the CAP, 100 mM tramadol (n = 7) was administered. An increase in the amplitude and threshold of the CAP was found at the intensity of 60 dB SPL (p = .012 repeated measure ANOVA). The effect was significant at 2, 20, and 40 min after the administration of tramadol. There were no consistent changes in CAP latency or threshold (Figure 5).

3.4 | Naloxone effects in ABR, CAP, and DPOAE

The effect of naloxone was studied by i.p. (10 mg/kg) in the ABR records. In the ABR registered using tone pip stimuli (1, 5, and 10 kHz) we found that naloxone i.p. (10 mg/kg) non-significantly increased the amplitude of PI and PII (especially at 1 kHz) (p = .08 for both components, n = 6) the other components of the ABR had no changes in amplitude or latencies.

In the CAP recordings, perfusion of 10 mM naloxone (n = 8) produced a decrement in the N1-P1 amplitude of the CAP which was significant after 40 min of the perfusion of naloxone (p = .031, Figure 6). The threshold of the CAP significantly increased after 40 min of the naloxone perfusion (p = .005). The latency of the CAP remained without any significant modification after the naloxone perfusion.

In the DPOAE recordings naloxone (10 mM, n = 9) administration produced a consistent decrease in the amplitude at mid frequencies f2 = 4.4 (p < .05) and at high frequency f2 = 17.4 kHz (p < .01), the effect was significant at all the intensity levels of the stimulus (from 35 to 80 dB SPL with f2 = 17.4 kHz, Figure 7). No changes were found in the DPOAE threshold after administration of naloxone.

4 | DISCUSSION

There is evidence in the literature demonstrating the expression of opioid peptides in synaptic complexes in the inner ear, as well as reports about the induction of sensorineural deafness due to the prolonged use of narcotic analgesics. It has been documented that the chronic use of
narcotic analgesics such as acetaminophen with hydrocodone, causes gradual sensory deafness requiring a cochlear implant for the recovery of hearing (Friedman, House, Luxford, Gherini, & Mills, 2000; Ho, Vrabec, & Burton, 2007; Lopez, Ishiyama, & Ishiyama, 2012; Prommer, 2005). The temporary loss of hearing produced by methadone, a synthetic opiate, has also been reported (Christenson & Marjala, 2010, Saifan, Glass, Barakat, & El-Sayegh, 2013, Van Gaalen, Compier, & Fogteloo, 2009). In studies in mouse cochlear cultures, it has been reported that hydrocodone would act synergistically with acetaminophen to induce cell death (Yorgason, Kalinec, Luxford, Warren, & Kalinec, 2010). To determine whether opioids exert an effect on the early auditory response, and to elucidate at what level of the auditory pathway they would be acting, we decided to study the preparation of the whole anesthetized animal and to record the ABR, the CAP, and the DPOAE. The ABR allows us to evaluate different levels of the auditory pathway, from the periphery to the central nuclei. The CAP evaluates cochlear responses exclusively, but more specifically. Finally, the DPOAE recording allows the evaluation of cochlear mechanics and the functionality of the cochlear amplifier. It should be noted that anesthesia produced by the ketamine—xylazine administration, as done in this work, has not been reported to induce significant changes in the amplitude or latency of the ABR, the CAP or the DPOAE components (Arai, 2008; Goss-Sampson & Kriss, 1991). Ketamine acts as an NMDA receptor antagonist (Mion & Villlevieille, 2013; Zanos et al., 2018), and xylazine is an α2 adrenergic receptor agonist.
Although the primary auditory afferent transmitter is glutamate, it has been reported that the ketamine–xylazine anesthesia does not affect the earlier components of auditory responses that represent the activity of the auditory nerve (Brown, Smith, & Nutall, 1983; Goss-Sampson & Kriss, 1991; Schwender, Klasin et al., 1993; Smith & Mills, 1989).

Our long term control recordings of anesthetized animals showed no significant time along 120 min recordings, independently of any potential central actions of the drugs used for anesthesia.

Naloxone at the dose reported by Sahley et al. (1991), did not produce a consistent effect on ABRs, except for a decrease in response latency. Morphine and fentanyl induced an increase in the amplitude of PI and PII waves of the ABR, so we inferred that this opioid would be exerting its function both in the cochlea and in the cochlear nucleus, where the presence of the MOR has been demonstrated by in situ hybridization studies (Mansour et al., 1994). These results differ from that reported in the literature regarding the absence of effects on the components of early ABRs, in response to opioids administered systemically in other animal models such as the guinea pig (Sahley et al., 1991) or the macaque Rhesus (Samra, Kruitak-Krol, Pohorecki, & Domino, 1985). This is probably because the experiments were done using different anesthetics and routes of opioid administration. For its part, fentanyl was the most potent of the opioid agonists tested, although the dose used was lower than that reported in the literature to induce immobility in rats, it agrees with that used by Schwender, Rimkus et al., (1993) in human patients to induce anesthesia, where they report that there are no changes in the components of the ABR.

The action of opioid agonists and of naloxone on CAP were coherent with a potentiating action of opioid receptors in the afferent response. Also, the increase in the amplitude of the DPOAE suggests a role of opioids in modulating the cochlear mechanics, most probably due to the expression of KOR in OHC as suggested previously (Vega, García-Garibay, Flores, & Soto, 2006). The opioid receptors are coupled to the inhibitory G protein, and among the effects that are triggered by the activation of these receptors, are the inhibition of voltage-dependent

**FIGURE 4** Effects of tramadol (10 mg/Kg, n = 8) in the ABR recordings. In (a), ABR in control (black trace) and after 20 min perfusion of tramadol (10 mg/Kg, gray trace). In (b), tramadol (gray bars) produced a reduction in the ABR threshold for mid frequencies (16, 22, and 32 kHz) and an increase of the threshold at 48 kHz ($p = .05$ paired Student t test). Black bars are the control for all conditions. In (c), the ABR latency was without changes after tramadol perfusion. In (d), ABR amplitude decreased for low frequencies (2 kHz, $p = .01$ paired Student t test), and increased for higher than 8 kHz frequencies ($p > .05$)

**FIGURE 5** Effect of tramadol (100 mM, n = 7) in the CAP recording evoked by a click at 60 dB SPL. In (a), typical CAP recording in control (black trace) and after 20 min perfusion of tramadol (gray trace). In (b), Tramadol produced a reduction in the CAP threshold which is not significant even after 40 min ($p > .05$). In (c), the CAP amplitude significantly increased after tramadol perfusion ($p = .012$ repeated measures ANOVA). In (d), CAP latency did not show any tendency after tramadol perfusion.

(Browning, Lawrence, Livingston, & Morris, 1982; Greene & Thurmon, 1988). Although the primary auditory afferent transmitter is glutamate, it has been reported that the ketamine–xylazine anesthesia does not affect the earlier components of auditory responses that represent the activity of the auditory nerve (Brown, Smith, & Nutall, 1983; Goss-Sampson & Kriss, 1991; Schwender, Klasin et al., 1993; Smith & Mills, 1989).
calcium currents (Seseña, Vega, & Soto, 2014). It has been found that the activation of κ-type opioid receptors decreases the amplitude of the calcium current present in rat CCE (Vega et al., 2006), and in the hair cells of the axolotl (Ambystoma tigrinum), in addition to decreasing the frequency of vestibular nerve discharge (Vega & Soto, 2003). We propose that naloxone is blocking the opioid receptors in the IHC, reducing neurotransmitter release, as suggested by the decrease in the amplitude of the CAP induced by naloxone.

The effects of morphine, fentanyl, and tramadol may be attributable to its predominant action on MOR that have been proposed to be located at postsynaptic level. The activation of the MOR can increase the discharge in a group of neurons and decrease it in others, that could

**FIGURE 6** Effect of naloxone (10 mM, n = 8) on the CAP characteristics. In (a), recording of the CAP under control conditions (black trace) and after 40 min perfusion of naloxone (gray trace). Dotted line = 0 μV. In (b), bar graph of the threshold for CAP. Perfusion of naloxone increased the hearing threshold after 40 min of its perfusion (p = .005, ANOVA for repeated measures). In (c), the amplitude of the CAP decreases significantly after 40 min after the perfusion of Naloxone (p = .03). In (d), the latency of CAP did not show significant changes

**FIGURE 7** Effect of 10 mM naloxone in the recording of DPOAE (n = 9). Graphs of the DPOAE obtained at 70 dB SPL. In (a), record the DPOAE, in control (black trace) and 40 min after perfusion of naloxone (gray trace). Inset shows the peak of the DPOAE amplitude. In (b), the threshold of DPOAE at several frequencies in control and after administration of naloxone. In (c–e), naloxone induced a decrease in DPOAE that is significant with f2 = 4.4 and 17.4 kHz, at 20 and 40 min after drug administration. Stimulus level = 60 dB SPL (p < .05 repeated measures ANOVA)
account for the variability of the effects produced by opioids. Likewise, it is known that endomorphin-1 (endogenous MOR agonist), inhibits the currents caused by ACh in rat IHC, and in hair cells of the frog saccule, acting directly on the cholinergic receptor, and in Xenopus oocytes to which the ACh α9/α10 receptor was transfected (Lioudyno et al., 2002; Matthews, Duncan, Zidanic, Michael, & Fuchs, 2005).

It is interesting to note that in all of our experimental series in around 20% opioid drugs produced a strong effect. This result coincides with what is found in opioid users, which among thousands of them there is a low percentage of "susceptible" subjects who suffer from auditory alterations (Oroei, Peyvandi, & Mokhtarinejad, 2018). The mechanism of toxicity of opioids has not been determined, but the improvement of some patients by cochlear implantation suggests isolated cochlear damage (Ho et al., 2007). The fact that opioid receptors are expressed in the inner ear confirms the idea that cochlear damage is involved in the ototoxic action of opioids, although additional knowledge about the distribution and function of the opioid receptor in the inner ear is required to fully understand its toxicity (Ho et al., 2007; Jongkamonwiwat et al., 2006). Of the drugs tested in this work, morphine, fentanyl, and tramadol are typical drugs of abuse. Fentanyl is often added to heroin or disguised as heroin.

The mechanism that induces deafness due to the use of opioids is not fully understood. The most commonly proposed theories to explain secondary hearing loss to opioid use are ischemic cochlear damage secondary to vasospasm or vasculitis of the branch of the spiral modiolar artery, genetic variation in liver metabolic enzymes, transport of proteins and receptors, and the direct effect of opioids on opioid receptors (Oroei, Peyvandi, & Mokhtarinejad, 2018).

Most opioids of abuse are MOR agonists. These receptors are found in the inner and OHCs, the spiral ganglion, and the nerve fibers. The activation of MOR can inhibit the calcium current due to the inhibition of adenylate cyclase as has been demonstrated in vestibular afferents (Seseña et al., 2014). The endogenous opioid peptides (endorphin and enkephalin) have an important role in the auditory neuromodulation and their overactivation can lead to hearing loss. Another possibility is that the opioid receptors when chronically exposed to agonists may be internalized, which would lead to an accumulation of intracellular calcium that would eventually lead to cellular damage. In neurons isolated from the rat cochlea, KOR agonists produce a significant inhibitory effect on potassium and calcium currents (De la Rosa Jiménez, 2009). Similarly, in cases where sudden hearing loss due to opioids has been reported, it is plausible to assume that altering the protective mechanism mediated by ACh and opioid peptides, increased glutamate release thus inducing excitotoxicity in afferents ganglion neurons, producing sensorineural deafness.

It should also be considered that opioids exert actions at other levels, particularly at the circulatory and respiratory levels. Audiometric studies and otacoustic emissions in humans indicate that the damage is at least partly important in the inner ear. It has been suggested that hearing loss is due to cochlear ischemia secondary to opioid-induced vasoconstriction by stimulation of endothelin-1 (Nguyen, Lopez, Ishiyama, & Ishiyama, 2014). Vasoconstriction secondary to the consumption of opioids can also induce central auditory damage (Polpathapee, Tuchinda, & Chiwapong, 1984). Another mechanism that may be participating in the genetic polymorphism that may be a predisposing factor in opioid-induced hearing loss, similar to what happens in other cases of ototoxicity. Allelic variants in enzymes that metabolize the liver and mutations of the MOR gene can be found in several responses to opioid drugs in individuals.

### 4.1 Conclusions

Our results indicate that at the systemic level it is possible to demonstrate the modulation of the auditory input by opioid receptor agonists and antagonists. The selectivity in the effect for certain frequencies is coherent with a localized innervation of the cochlea by opioidergic efferents (Raphael & Altschuler, 2003). The results obtained do not allow us to discern about the distribution and characteristics of the opioid receptors that are participating in the effect we observed, but they suggest the presence of different subtypes of receptors with opposite effects as has been described in the vestibular systems (De la Rosa Jiménez, 2009; Vega et al., 2006). Regardless of the need to discern more precisely the subtypes of receptors involved in opioid modulation. The responses produced by the agonists are of low intensity as expected in a complex system such as the cochlea and which is subject to multiple modulatory inputs mediated by various neurotransmitters. Our results demonstrate for the first time that the activation of opioid receptors in the rat in vivo produce an excitatory modulation of the afferent discharge, and also modulate the cochlear amplifier.

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### CONFLICT OF INTEREST

The authors declare no competing financial interests.
DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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