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Excitatory and inhibitory effects of opioid agonists on respiratory motor output produced by isolated brainstems from adult turtles (*Trachemys*)

Stephen M. Johnson*, Christina M. Moris, Michelle E. Bartman, Liana M. Wiegel

Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin, 2015 Linden Drive, Madison, WI 53706, USA

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ABSTRACT

To determine how central opioid receptor activation alters turtle breathing, respiratory-related hypoglossal (XII) motor bursts were recorded from isolated adult turtle brainstems during 60 min bath applications of agonists for delta- (DOR), kappa- (KOR), or nociceptin/orphanin (NOR) receptors. DADLE (DOR agonist) abolished XII burst frequency at 0.3–0.5 μM. DPDPE (DOR agonist) increased frequency by 40–44% at 0.01–0.1 μM and decreased frequency by 88 ± 8% at 1.0 μM. U-50488 and U-59693 (KOR agonists) decreased frequency by 65–68% at 100 and 50 μM, respectively. Orphanin (NOR agonist) decreased frequency by 31–51% at 1.0–2.0 μM during the first 30 min period. Orphanin (0.5 and 2.0 μM) increased bursts/episode. Although morphine (10 μM) abolished frequency in nearly all brainstems, subsequent co-application of phenylephrine (α_1-adrenergic agonist, 20–100 μM) with morphine restored activity to 16–78% of baseline frequency. Thus, DOR, KOR, and NOR activation regulates frequency and NOR activation regulates episodicity, while α_1-adrenergic receptor activation reverses opioid-induced respiratory depression in turtles.

1. Introduction

Opioid drugs are clinically administered to relieve pain, as well as reduce coughing, diarrhea, and anxiety. However, opioid drugs have deleterious side effects, such as respiratory depression, nausea and vomiting, drowsiness, dry mouth, hypotension, and constipation (Petti and Arndt, 1993; Inturrisi, 2002). In mammals, opioid-dependent respiratory depression is due to decreased respiratory frequency, tidal volume, and chemoreceptor drive, as well as increased upper airway resistance and altered pulmonary mechanics (Santiago and Edelman, 1985; Shook et al., 1990; Babenco and Gross, 1993). Opioid-dependent changes in breathing are primarily due to opioid receptor activation on brainstem respiratory neurons (Denavit-Saubie et al., 1978; Rondouin et al., 1981; Takita et al., 1997; Gray et al., 1999; Takeda et al., 2001; Lalley, 2003, 2006). However, systemic opioid administration also activates peripheral opioid receptors in the lungs that modulate pulmonary reflexes and alter cardiorespiratory function (Willette and Sapru, 1982a,b; Willette et al., 1982). Likewise, opioid receptor activation in the carotid body inhibits chemoreceptor afferent discharge (McQueen and Ribeiro, 1980; Kirby and McQueen, 1986).

In contrast, far less is known about the opioid drug effects on breathing in non-mammalian ectothermic vertebrates, such as jawless fish, amphibians, and reptiles. Mu-opioid (MOR) and delta-opioid (DOR), but not kappa-opioid (KOR), activation reduces respiratory burst frequency without altering burst pattern in isolated lamprey brainstems (Mutolo et al., 2007). In intact frogs, morphine (primarily MOR agonist with DOR and KOR activity) reduces lung ventilation frequency, lung episode frequency, and the number of lung breaths/episode (Vasilakos et al., 2005, 2006). In reptiles, such as red-eared slider turtles (*Trachemys scripta*), morphine and butorphanol (mixed KOR agonist and MOR agonist/antagonist) depresses ventilation due to a 60–80% reduction in breathing frequency (Sladky et al., 2007). This respiratory depression appears to be due to MOR and DOR activation since systemic administration of specific MOR or DOR agonists decrease ventilation by decreasing breath frequency (Johnson et al., 2008). On the other hand, KOR activation produces highly variable effects on breath frequency (some turtles stop breathing for min to h) and increases tidal volume in time-dependent manner (Johnson et al., 2008). Given that systemic drug administration in intact animals may also alter the function of peripheral chemo- and mechanoreceptors, it is important to test how central opioid receptor activation alters respiratory motor output. In isolated turtle brainstems (Johnson et al., 2002) and hemibrainstems (Majewski et al., 2008), MOR activation abolishes respiratory motor output, but the effects of DOR or KOR activation *in vitro* are not known. Furthermore, only one study showed that NOR activation in neonatal rat brainstem–spinal cord preparations decreased respiratory frequency with little effect on spinal respiratory motor output

* Corresponding author. Tel.: +1 608 261 1104; fax: +1 608 263 3926.
E-mail address: johnsons@svm.vetmed.wisc.edu (S.M. Johnson).

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amplitude (Takita et al., 2003). Thus, the role of NOR activation in adult vertebrate respiratory motor control is not known.

Finally, respiratory depression is the most significant adverse side effect of opioid drugs in human and veterinary clinical medicine (Pattinson, 2008). Consequently, several pharmacological methods for reversing opioid-induced respiratory depression have been developed in mammalian experimental preparations. For example, opioid-induced respiratory depression is reversed while maintaining analgesia by co-administering opioid drugs with a serotonin 5-HT_4 agonist (Manzke et al., 2003), dopamine D_1 agonist (Lalley, 2005), cholinesterase inhibitor (Weinstock et al., 1980), ampakine (Ren et al., 2006), or DOR agonist drug (Su et al., 1998). For reptiles and other non-mammalian vertebrates, however, there are no studies testing whether opioid-induced respiratory depression can be reversed. Since α_1-adrenergic receptor activation augments respiratory frequency (Johnson et al., 1998) and induces long-lasting frequency increases in isolated turtle brainstems (Wilkerson et al., 2003b), α_1-adrenergic agonists are candidate drugs for reversing opioid-induced respiratory depression in reptiles.

To address these questions, adult turtle brainstems were isolated under *in vitro* conditions, spontaneous respiratory motor output on hypoglossal (XII) nerve roots was recorded, and brainstems were exposed to specific opioid receptor agonists. *In vitro* turtle brainstems are advantageous because inspiratory- and expiratory-related motor output is produced that is qualitatively similar to that produced by intact turtles (Douse and Mitchell, 1990; Johnson et al., 1998). Also, turtle brainstems are extremely resistant to hypoxia (Johnson et al., 1998; Jackson, 2000), which means that respiratory-related motor output can be produced by fully mature turtle brainstems for several days at physiologically relevant temperatures (Wilkerson et al., 2003a). We hypothesized that brainstem DOR and NOR activation would decrease respiratory burst frequency without altering burst amplitude, while brainstem KOR activation would have no effects. Surprisingly, we found that DOR had mixed, dose-dependent excitatory and inhibitory effects on respiratory burst frequency, KOR decreased burst frequency, and NOR activation increased the number of bursts/episode (episode = two or more respiratory motor bursts occurring sequentially followed by a pause). Finally, we found that α_1-adrenergic receptor activation reversed morphine-induced respiratory depression in isolated turtle brainstems.

2. Methods

All procedures were approved by the Animal Care and Use Committee at the University of Wisconsin-Madison School of Veterinary Medicine. Adult red-eared slider turtles (*T. scripta*, $n = 155$; 750 ± 11 g) were obtained from commercial suppliers and kept in large open tanks where they had access to water for swimming, and heat lamps and dry areas for basking. Room temperature was set to 27–28 °C with light 14 h/day. Turtles were fed ReptoMin® floating food sticks (Tetra, Blacksburg, VA) 3–4 times per week.

2.1. Turtle brainstem preparations

Turtles were intubated and anesthetized with 5% isoflurane (balance oxygen) until head and limb withdrawal reflexes were eliminated, upon which the turtles were decapitated. The brainstem was removed and pinned onto Sylgard® in a recording chamber (5 ml volume). The tissue was superfused (4–5 ml/min) with artificial cerebrospinal fluid (aCSF) containing HEPES (N-[2-hydroxyethyl]piperazine-N′-[2-ethane-sulfonic acid]) buffer as follows (mM): 100 NaCl, 23 $NaCHO_3$, 10 glucose, 5 HEPES (sodium salt), 5 HEPES (free acid), 2.5 $CaCl_2$, 2.5 $MgCl_2$, 1.0 K_2PO_4, 1.0 KCl. The HEPES solution was bubbled with 5% CO_2–95% O_2 to maintain pH 7.34 ± 0.01, as measured periodically with a calomel glass pH electrode (Cole-Parmer Inst. Co., Vernon Hills, IL, USA). The low pH of the aCSF (normal turtle arterial pH = 7.6) is necessary to "drive" turtle brainstems to produce respiratory motor output that has an amplitude and frequency that permits experiments to be performed in a timely manner (see Johnson et al., 1998).

2.2. Electrophysiological recordings

Glass suction electrodes were attached to XII nerve rootlets (Fig. 1). Signals were amplified (10,000×) and band-pass filtered (1.0–500 Hz) using a differential AC amplifier (model 1700, A-M Systems, Everett, WA, USA) before being rectified and integrated (time constant = 200 ms) using a moving averager (MA-281/RSP, CWE, Inc., Ardmore, PA, USA). The signals were digitized (50 Hz) and analyzed using Axoscope software (Axon Instruments, Foster City, CA, USA) and MiniAnalysis software (Synaptosoft, Inc., Decatur, GA, USA).

2.3. Drugs

The following drugs were obtained from Sigma/RBI Aldrich (St. Louis, MO): DADLE (DOR agonist; [D-Ala^2, D-Leu^5]-Enkephalin acetate salt), DPDPE (DOR agonist; [D-Pen^{2,5}]-Enkephalin hydrate), U-50488 (KOR agonist; (−)-trans-(1S,2S)-U-50488 hydrochloride hydrate), U-69593 (KOR agonist; (+)-(5α,7α,8β)-N-Methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-benzeneacetamide), orphanin (NOR agonist; Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln); and phenylephrine (α_1-adrenergic receptor agonist). Morphine sulfate was obtained from Baxter Healthcare Corporation.

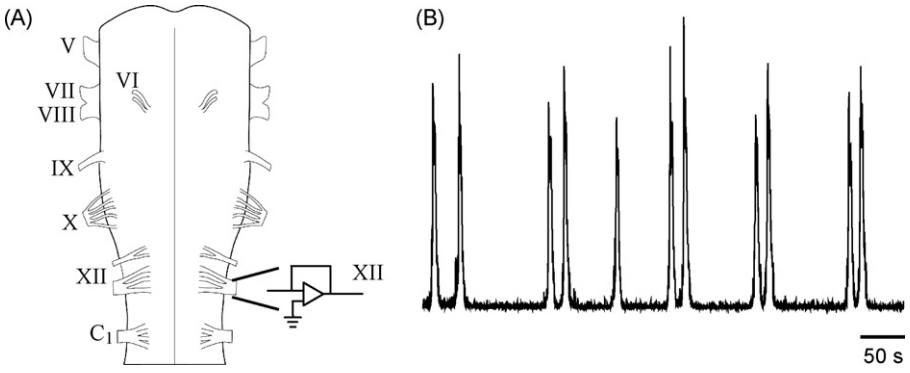

Fig. 1. Isolated turtle brainstem produces spontaneous respiratory motor output. (A) Drawing of turtle brainstem with cranial nerves marked as shown on the left. A suction electrode attached to the hypoglossal (XII) nerve records respiratory motor output. (B) Trace of integrated and rectified respiratory motor output demonstrating both singlet and doublet XII motor bursts produced by the same brainstem.