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Original Reports

Aconitum-Derived Bulleyaconitine A Exhibits Antihypersensitivity Through Direct Stimulating Dynorphin A Expression in Spinal Microglia

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Abstract: Aconitine and its structurally-related diterpenoid alkaloids have been shown to interact differentially with neuronal voltage-dependent sodium channels, which was suggested to be responsible for their analgesia and toxicity. Bulleyaconitine A (BAA) is an aconitine analogue and has been prescribed for the management of pain. The present study aimed to evaluate the inhibitory effects of BAA on pain hypersensitivity and morphine antinociceptive tolerance, and explore whether the expression of dynorphin A in spinal microglia was responsible for its actions. Single intrathecal or subcutaneous (but not intraventricular or local) injection of BAA blocked spinal nerve ligation-induced painful neuropathy, bone cancer-induced pain, and formalin-induced tonic pain by 60 to 100% with the median effective dose values of 94 to 126 ng per rat (intrathecal) and 42 to 59 μg/kg (subcutaneous), respectively. After chronic treatment, BAA did not induce either self-tolerance to antinociception or cross-tolerance to morphine antinociception, and completely inhibited morphine tolerance. The microglial inhibitor minocycline entirely blocked spinal BAA (but not exogenous dynorphin A) antinociception, but failed to attenuate spinal BAA neurotoxicity. In a minocycline-sensitive and lidocaine- or ropivacaine-insensitive manner, BAA stimulated the expression of dynorphin A in the spinal cord, and primary cultures of microglia but not of neurons or astrocytes. The blockade effects of BAA on nociception and morphine tolerance were totally eliminated by the specific dynorphin A antiserum and/or κ-opioid receptor antagonist. Our results suggest that BAA eliminates pain hypersensitivity and morphine tolerance through directly stimulating dynorphin A expression in spinal microglia, which is not dependent on the interactions with sodium channels.

***Perspective:** The newly illustrated mechanisms underlying BAA antinociception help us to better understand and develop novel dynorphin A expression-based painkillers to treat chronic pain.*

***Key words:** Bulleyaconitine A, anti-hypersensitivity, morphine tolerance to antinociception, spinal microglia, dynorphin A, sodium channel.*

For centuries, preparations of the *Aconitum* genus have been used for analgesic, antirheumatic and neurological indications in China and other Asian countries. As a principal group of compounds present in *Aconitum*, 170 alkaloids have been identified and classified into 4 categories: C20-, C19-, C18-, and *bis*-diterpenoid alkaloids.[2,14] The C19-diterpenoid group represented by aconitine comprises the most toxic alkaloids. Bulleyaconitine A (BAA), isolated from *Aconitum bulleyanum*, belongs to the "aconitine-like" alkaloids but differs from aconitine with 2 hydroxyl groups at C3 and C15 and a *p*-methoxy-benzoylester group at C14.[72] The chemical structures of aconitine and BAA are presented in Fig 1. Because it has lower toxicity and a wider therapeutic window than aconitine,[4,20] BAA was introduced into clinic in China for the treatment of chronic pain for 3 decades.[66] Administration of BAA blocked acetic acid- and formalin-induced pain, with a

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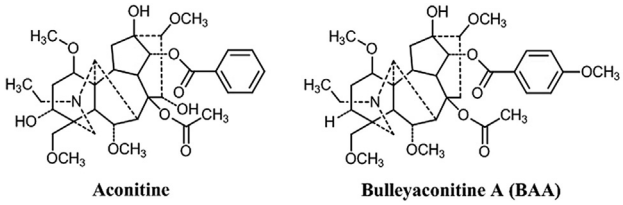

Figure 1. The chemical structures of aconitine and its analogue, BAA.

65- and 7,200-fold greater potency than morphine and aspirin, respectively.[66] BAA is classified as a non-narcotic analgesic, and no physical dependence has been observed in monkeys.[86]

The mechanism underlying BAA analgesia is unclear, although some studies have postulated that its blockage of neuronal voltage-dependent sodium (Nav) channels is responsible. BAA blocked Na^+ currents, particularly the Nav1.7 and Nav1.8 Na^+ currents, by more than 90% in a use-dependent manner.[71,72] The blockade of Nav1.7 and Nav1.8 sodium channels was antinociceptive.[7,40] Lappaconitine and N-deacetyl-lappaconitine had similar blockage effects on sodium channels.[57] In contrast, aconitine, the main diterpenoid alkaloid in *Aconitum* plants, and acetylaconitine bound with high affinity to the open state of sodium channels, causing its persistent activation of sodium channels by blocking inactivation and eventually leading to inexcitability.[3,9] The structure–activity relationship analysis established a strong correlation between the toxicity and analgesic activity of aconitine and its analogues.[4] Aconitine and its alkaloids have generally been believed to interact with neuronal sodium channels and be responsible for their analgesia and toxicity,[2,14] although there is no direct causal evidence provided for their analgesia.

However, the relatively wide therapeutic window of BAA suggests that its antinociception could be separated from its toxicity[20] and was possibly due to mechanisms other than interference with sodium channels. The antinociceptive effects of *Aconitum* alkaloids (BAA, mesaconitine, 3-acetylaconitine, and lappaconitine) were shown to be due to the activation of the descending inhibitory system transmitted by norepinephrine and serotonin,[44,50,66,86] and the influence of the noradrenergic system on aconitine and 3-acetylaconitine antinociception was mediated by the inhibition of sodium channel–dependent norepinephrine uptake.[57] In contrast, the antinociceptive effects of the *Aconitum* extracts were mediated by the stimulation of dynorphin A release and activation of κ-opioid receptors.[49,63,77] The underlying upstream mechanisms are unknown, especially initial targeting cell types in the central nervous system and the relationship with sodium channels.

Spinal microglia play a crucial role in the development of chronic pain.[16] We recently showed that activation of glucagon-like peptide-1 (GLP-1) receptors stimulated β-endorphin expression in spinal and hippocampal microglia, leading to antinociception and neuroprotection, and the Tibetan herbal analgesic *Lamiophlomis* rotata and herbal catalpol produced antinociception and neuroprotection by activating the spinal and hippocampal GLP-1 receptor/β-endorphin pathway.[18,19,21,24,29,87] We hypothesized that BAA produced antinociception by stimulating dynorphin A expression in spinal microglia, which was separate from the blockage of sodium channels that presumably mediated neurotoxicity.

Methods

Drugs and Reagents

BAA was purchased from Zelang Bio-Pharmaceutical (Nanjing, China) and ropivacaine mesylate was obtained from Xi'an Libang Pharmaceutical Co (Xi'an, China). Morphine hydrochloride, lidocaine, and minocycline were purchased from Northeast Pharmaceuticals Group (Shenyang, China), Chengdu First Pharmaceuticals Group (Chengdu, China), and Yuanye Biotech (Shanghai, China), respectively. CTAP and nor-binaltorphimine dihydrochloride (nor-BNI) were obtained from Abcam (Cambridge, United Kingdom) and naltrindole from Tocris Bioscience (Bristol, United Kingdom). The rabbit polyclonal antibodies neutralizing dynorphin A and β-endorphin were purchased from Phoenix Pharmaceuticals (Burlingame, California) and Abcam, respectively. On the basis of the manufacturers' information, the dynorphin A antiserum was specific to dynorphin A (100%), but not to dynorphin B (0%), β-endorphin (0%), α-neo-endorphin (0%), or leu-enkephalin (0%); the β-endorphin antiserum was specific to β-endorphin and did not cross-react with methionine-enkephalin, leucine-enkaphalin, dynorphin A or B, γ-endorphin, α-endorphin, adrenocorticotropic hormone, or α-melanocyte–stimulating hormone. The specificities of the dynorphin A antiserum[70,79] and β-endorphin antiserum[62] were also validated by using the antigen absorption tests from other laboratories. All of the drugs and reagents were dissolved or diluted in .9% normal saline.

Experimental Animals

Male and female adult (200 ± 20 g body weight) and 1-day-old neonatal Wistar rats were obtained from the Shanghai Experimental Animal Institute for Biological Sciences (Shanghai, China). The animals were housed in plastic cages with 4 per cage and thick sawdust bedding at standard room temperature (22 ± 2°C), under conditions of a 12/12-hour reversed light-dark cycle (7:00 AM–7:00 PM), and received food and water ad libitum. They were accustomed to the laboratory environment for 3 to 5 days before the experiments. Experimental study groups (n = 6 per group except for the BAA neurotoxicity study in which there were 12 per group) were randomly assigned, and the researchers (T.F.L., H.F., Y.X.W.) were blinded for the behavior tests. The research protocols were approved and performed in accordance with the Animal Care and Welfare Committee of Shanghai Jiao Tong University and carried out in accordance with the Animal Care Guidelines of the National Institutes of Health.

Primary Neuronal and Glial Cell Culture

The 1-day-old neonatal rats and adult rats (180 ± 20 g body weight) were decapitated under anesthesia