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Research report

Effects of microinjections of neurotoxin AvTx8, isolated from the social wasp *Agelaia vicina* (*Hymenoptera, Vespidae*) venom, on GABAergic nigrotectal pathways

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Abstract

Several investigations have provided information that defensive behaviors evoked by stimulation of deep layers of the superior colliculus (dlSC) are subjected to inhibitory nigral modulation. This inhibition is made mainly through GABAergic neurons from substantia nigra, pars reticulata (SNpr), that sends outputs toward neural networks of the deep layers of the superior colliculus and dorsal periaqueductal gray matter involved with the organization of fear-like responses. In this work, we compared the effects of two GABAergic agonists, muscimol and baclofen, with the effect of neurotoxin AvTx8 (1567 Da), isolated from the venom of the social wasp *Agelaia vicina*, microinjected into SNpr of *Rattus norvegicus* (Wistar rats) prior to dlSC saline or bicuculline microinjections, considering that wasp venom has some influence on the uptake of GABA and/or glutamate neurotransmitters. $GABA_A$ receptor blockade in the dlSC evoked a vigorous escape behavior, expressed by rapid running, jumps and turns, as compared to control. These defensive reactions were maximized after the intranigral $GABA_A$ agonism with muscimol, but not after in situ $GABA_B$ agonism. Nigral microinjection of AvTx8 induced similar effects to those of baclofen, decreasing the intensity of behavioral defensive reactions caused by $GABA_A$ receptor blockade in the dorsal mesencephalon. These findings suggest that AvTx8 has some effects on GABAergic neurotransmission, increasing the activity of the inhibitory nigro-collicular pathways, causing an anti-panic (antiaversive) effect. Therefore, our work suggests AvTx8 as a novel pharmacological tool to study differences between the two types of GABAergic receptors and excitatory amino acid-mediated mechanisms in the brain and brainstem networks.

Theme: Neurotransmitters, modulators, transporters and receptors
Topic: Behavioral pharmacology

Keywords: Wasp venom; Substantia nigra; $GABA_A$ receptor; $GABA_B$ receptor; GABAergic striato-nigro-tectal pathways; Excitatory amino acid-mediated mechanisms

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1. Introduction

There is evidence that neurons of the substantia nigra, pars reticulata (SNpr), exert an important modulatory influence on the neural network of the dorsal mesencephalon involved in the generation and organization of fear- and panic-like responses [9,8,13]. In fact, the defensive reactions elicited by electric and chemical stimulation of the mesencephalic tectum have been proposed as a useful model for studying the panic-induced behavior in animals [41,40,2], considering previous evidences suggesting the involvement of midbrain structures in fear- and pain-related reactions in humans [16,26,38].

The substantia nigra is a mesencephalic structure with a very high density of GABAergic circuits, functionally linked to the basal nuclei, which comprises the neostriatum (nucleus caudatus and putamen), globus palidus and subthalamic nucleus [11,12,33,34,18,17,19], and receives GABAergic projections from the neostriatum [17,8]. However, recent functional neuroanatomical evidence has been provided for a GABAergic nigrotectal output from SNpr targeting the deep layers of the superior colliculus (dlSC) and both dorsomedial and dorsolateral columns of the periaqueductal gray matter (dmPAG/dlPAG), modulating the organization of panic-like behaviors [13]. These findings corroborated previous reports suggesting that striado-nigro-collicular pathways exert a tonic GABAergic inhibitory control on the neuronal network of the dorsal mesencephalon involved with the elaboration of motor reactions and defensive behavior [9,8,36].

In fact, several investigations have reported that electrical or chemical stimulation of midbrain tectum structures, such as dmPAG/dlPAG, dlSC and inferior colliculus, elicit, in a progressive manner, aversive responses characterized by defensive attention (alertness), defensive immobility (freezing) and escape behavior [4,6,3,8,10,27]. This set of behaviors are either exacerbated or inhibited by intranigral injections of GABA agonists or antagonists, respectively [8]. Since the responses are clearly linked to GABAergic circuitry, SNpr could be an interesting neuroanatomical target to verify the neurobiological activity of a neuroactive toxin that could interfere with the GABA or glutamatergic neurotransmission.

To this date, a great number of neurotoxic compounds from animal venoms have been identified [5,35,42,24,15] in a variety of molecular classes, such as acylpolyamines and polypeptides [1,20–23,25,32]. Some of these toxins are highly selective to mammalian nervous tissues, being widely used in a great number of investigations of neuronal mechanisms [14,20,29,39].

In one of these reports, Pizzo and colleagues [30] demonstrated that the deproteinated venom from *A. vicina* strongly inhibits, in an uncompetitive manner, the uptake of GABA and L-Glu in synaptosomes from Wistar rat cerebral cortex. This work led to a further purification of two neurotoxins (AvTx7 and 8) with remarkable activities in rat cerebrocortical synaptosomes [31].

Therefore, the present study aimed to examine the behavioral effects of the neurotoxin AvTx8, isolated from the *A. vicina* venom, microinjected into the substantia nigra, pars reticulata, in non-anesthetized Wistar rats pretreated with bicuculline in the dlSC. The effects of AvTx8 were compared to those of $GABA_A$ and $GABA_B$ agonists also microinjected into the substantia nigra, in the same animal model.

2. Materials and methods

2.1. Wasp toxin

Workers of *A. vicina* were collected in Ribeirão Preto, São Paulo, Brazil, killed by rapid freezing and stored at −20 °C. Venom sacs were then dissected, washed in isotonic water and manually crushed using a glass pestle containing 1:1 acetonitrile/water, and centrifuged for 3 min at 1000×*g*. Venom was then purified as described by Pizzo and colleagues [31].

2.2. Animals

Male Wistar rats, weighing 230–250 g, from the animal facility of the Campus of Ribeirão Preto of the University of São Paulo, were housed in individual Plexiglas-walled cages under a 12:12-h dark/light cycle (lights on at 07:00 h) at 23±1 °C and given free access to food and water. All protocols of this study were used according to the rules for animal experimentation of the SBNec (Brazilian Society of Neuroscience and Behavior).

2.3. Surgical procedure

The animals were anesthetized with sodium thiopental 40 mg/kg (Cristália, Brazil) and were stereotaxically implanted with two guide cannulas. One stainless steel guide-cannula (outer diameter 15 mm, inner diameter 0.4 mm) was unilaterally implanted into the midbrain, aimed at the deep layers of the superior colliculus (dlSC), and another guide cannula with same size was implanted into the midbrain, aimed at the substantia nigra, pars reticulata (SNpr), following the coordinates from the Paxinos and Watson's atlas [28]: anteroposterior, −5.8 mm; mediolateral, 1.2 mm to dlSC and 2.0 mm to SNpr; and dorsoventral, 4.6 mm to dlSC and 7.5 mm to SNpr. Both cannulas were attached to the skull with acrylic resin, anchored with stainless steel screws and temporarily sealed with a stainless steel wire to protect them from obstruction.

2.4. Apparatus and behavioral procedure

One week after surgery, rats (n=8 per group) were placed in an arena (circular enclosure 60 cm in diameter and 50 cm high) with the floor divided in 12 sections for habituation. Afterwards, the animals were microinjected with the aid of a