Neuroscience Letters 509 (2012) 60–63

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Effects of microinjections of apomorphine and haloperidol into the inferior colliculus on prepulse inhibition of the acoustic startle reflex in rat

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ARTICLE INFO

Article history:
Received 24 September 2011
Received in revised form
20 December 2011
Accepted 23 December 2011

Keywords:
Apomorphine
Haloperidol
Inferior colliculus
Prepulse inhibition
Rat
Schizophrenia

ABSTRACT

Prepulse inhibition (PPI) is the reduction in the startle response caused by a low intensity non-startling stimulus (prepulse) which is presented shortly before the startle stimulus and is an operational measure of sensorimotor gating. PPI is impaired in schizophrenia patients and in rats with central dopamine (DA) activation. The inferior colliculus (IC) is a critical part of the auditory pathway mediating acoustic PPI. The activation of the IC by the acoustic prepulse reduces startle magnitude. The aim of this study was to elucidate the role of DA transmission of the IC on the development of acoustic PPI. Bilateral microinjections of apomorphine (9.0 μg/0.5 μL), an agonist of D_2 receptors, into the IC disrupted PPI while microinjections of haloperidol (0.5 μg/0.5 μL), an antagonist of D_2 receptors, into this structure did not alter PPI. These results suggest that dopamine-mediated mechanisms of the IC are involved in the expression of PPI in rodents.

1. Introduction

The acoustic startle response is evoked by a sudden and loud acoustic stimulus and is expressed as a rapid contraction of the facial and skeletal muscles. The magnitude of the acoustic startle response can be reduced by a relatively weak sound (prepulse) presented immediately before the startle-eliciting sound [8,11,19,21,25]. This phenomenon has been termed prepulse inhibition (PPI) of the acoustic startle response (ASR), and may reflect the functioning of a pre attention filtering system protecting the brain from sensory overload [11,14]. Deficits in PPI have been observed in several neuropsychiatric disorders, including schizophrenia [11], obsessive-compulsive disorder [23], Huntington's disease [24] and Tourette syndrome [4]. These deficits may be linked to impairments in sensorimotor gating a mechanism that enables normal individuals to suppress or "gate" irrelevant or interfering information in sensory, cognitive and motor domains, which allows the hierarchical organization of the most relevant information [7,11,14]. PPI provides an important operational measure of sensorimotor gating. This justified the study of PPI for a better understanding of the pathophysiology of schizophrenia and aid in the development of new therapies.

Previous studies indicated that the primary neural pathways mediating PPI is in the brain stem and that the inferior colliculus (IC) was crucial [14,22]. The central nucleus of the IC receives auditory input, which is relayed to the external nucleus of the IC before going to the middle layers of the superior colliculus (SC). In turn, the SC sends bilateral projections to the pedunculopontine tegmental nucleus (PPTg) [1,8,10]. The transient activation of these midbrain nuclei by the prepulse is converted into long-lasting inhibition of the giant neurons of the caudal pontine reticular nucleus (PnC) thus reducing startle response [8]. Large lesions of the IC eliminated the inhibition of acoustic startle by auditory but not by visual prepulses [8,13,20]. Electrical stimulation of the IC before the acoustic startle stimulus attenuated PPI in rats without major effects on startle amplitude [14,22]. Therefore, the IC is a critical part of the auditory pathway mediating acoustic PPI [8,10].

PPI deficits similar to those seen in schizophrenia patients can be induced in rats by systemic administration of a direct or an indirect dopaminergic (DA) agonist, N-methyl-D-aspartate (NMDA) glutamatergic antagonist, and direct or an indirect serotonergic (5HT) agonist [11]. More typically, deficits in PPI are induced by the administration of a dopamine agonist, such as apomorphine, with attenuation of the apomorphine-induced deficit representing a means by which potential antipsychotic agents can be evaluated [11]. Apomorphine has been reported to be a full agonist of D_2 receptors [11]. The disruption in PPI caused by DA agonists is reversed by the typical antipsychotic haloperidol, a drug with high D_2 receptor-binding affinity. Although apomorphine and haloperidol are 'D2-prefering' drugs, they also have a relatively high D1

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doi:10.1016/j.neulet.2011.12.052

affinity. Haloperidol is considered a classical antypsichotic acting primarily as DA antagonists. The presence of D_2 receptors in the IC has been reported using techniques such as autoradiography after labeling with highly selective ligands, *in situ* hybridization, and northern blot analysis [5,16,26].

Thus, the purpose of the present study was to elucidate the role of dopaminergic transmission in the IC on the expression of acoustic PPI. For that we investigated whether bilateral microinjections of apomorphine or haloperidol into the IC would affect PPI.

2. Materials and methods

2.1. Animals

A total of 29 naïve male Wistar rats provided by CEDEME – Federal University of Sao Paulo weighing 250–300 g at the beginning of the experiments were used for all experiments. They were housed in individual Plexiglas-wall cages in a 12:12 dark/light cycle (lights on at 07:00 am) under standard conditions in a temperature ($22 \pm 1\,^{\circ}C$) and humidity ($55 \pm 5\%$) controlled room with food and water given *ad libitum* for the extent of the study. The experiments were conducted during the light phase of the light/dark cycle, between 12:00 and 18:00 h. The experiments reported in this study were performed in compliance with the recommendations of SBNeC (Brazilian Society for Neuroscience and Behavior), which are based on the US National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) and were approved by the ethics committee of the Federal University of Sao Paulo (801/09). All efforts were made to minimize the number of animals used and their suffering.

2.2. Drugs

Apomorphine hydrochloride at a concentration of 9.0 μg/0.5 μL (Sigma, USA) was dissolved in distilled water [2,16]. Haloperidol (Janssen, Belgium) was prepared from 5 mg ampoules, in which the drug is present in 1 mL of vehicle solution containing 6 mg lactic acid. This solution was subsequently diluted with physiological saline to obtain the required concentration of 0.5 μg/0.5 μL [16]. These doses were based on previous studies [16]. The injections were done bilaterally. Drug solutions were freshly prepared before administration. Distilled water or physiological saline served as vehicle control for IC microinjections.

2.3. Startle chambers

Two commercial startle chambers devices (Insight Equipment, Brazil) were used simultaneously to record the amplitude of the acoustic startle response in the PPI test. The equipment consisted of a wire-mesh cage (16.5 cm × 5.1 cm × 7.6 cm) which was connected to a stabilimeter (response platform, 36.5 cm × 11.5 cm × 4.5 cm) with four thumb nail-screws, inside a ventilated, sound-attenuated chamber (48 cm × 48 cm × 45 cm). Noise bursts were presented *via* a high-frequency loudspeaker located 24 cm from the wire-mesh cage. The startle reaction of the rat within the wire-mesh cage generated a pressure on the stabilimeter, and signals were amplified, digitized and analyzed by the software of the startle measurement system (Insight Equipment, Brazil), and interface assembly, which also digitized, and recorded stabilimeter readings. Calibrations were performed weekly to maintain accurate acoustic stimuli presentations and ensure equivalent sensitivities of the response platforms over the test sessions. Animal behavior was recorded by an infrared camera (Safety View) located behind the stabilimeter, allowing the discrimination of all possible behaviors, with the signal being relayed to a video and a monitor in another room *via* a closed circuit.

2.4. Procedures

2.4.1. Surgery

The animals were anesthetized with sodium pentobarbital (45 mg/kg, ip) and fixed in a stereotaxic frame (David Kopf, USA). The upper incisor bar was set 3.3 mm below the interaural line, such that the skull was horizontal between bregma and lambda. A stainless steel guide cannula (o.d. 0.6 mm, i.d. 0.4 mm) was introduced vertically bilaterally, aimed at the IC using the following coordinates, with the bregma serving as the reference for each plane: antero-posterior = −8.8 mm; medio-lateral = 1.5 mm and dorso-ventral = 3.5 mm [17]. The guide cannula was affixed to the skull with acrylic resin and two stainless steel screws. A stylette inside each guide cannula prevented obstruction. All subjects were allowed a period of 7 days of recovery after surgery with *ad libitum* access to food and water.

2.4.2. Baseline startle session

One week after surgery, all rats underwent a brief baseline startle/PPI session consisting of 120 dB pulse-alone trials and prepulse + pulse trials in which a prepulse stimulus, 12 dB above background noise, was presented 100 ms before the onset of the 120 dB pulse. After a 5 min period of acclimation in the startle chamber, with a constant background noise (65 dB) that continued throughout the remainder of the session, a total of 24 trials were presented in a pseudorandom order: 18 presentations of a 40 ms, 120 dB broadband burst and 6 trials in which a 77 dB, 20 ms burst preceded the 120 dB burst by 100 ms. The experimental groups were established by using the mean startle response to the 120 dB pulse-alone trials and the mean %PPI calculated from the prepulse + pulse trials (see formula in Section 2.5), so that all groups had comparable baseline startle reactivity and PPI reducing inter-group variability. The day after the baseline session, drug testing took place [18].

2.4.3. Microinjections

After removal of the stylette, microinjections were made using thin dental needle stainless steel cannulae (Mizzy, o.d. 0.3 mm) introduced bilaterally through the guide cannulae until their lower ends were 1 mm below the guide cannulae. Each infusion cannula was connected to a 10 μL Hamilton syringe by polyethylene tubing, and a volume of 0.5 μL of vehicle or drug solution was delivered simultaneously into each IC over 1 min by an infusion pump (Insight Equipment, Brazil). The needle was left in place for an additional 1 min after injection. After that, the stylette was replaced. The rats were placed in the startle chambers immediately after the injection.

2.4.4. Testing startle and PPI

Immediately after vehicle or drug microinjections, animals were tested in the acoustic startle/PPI paradigm. The acoustic startle session consisted of a 5 min acclimation period in the startle chamber with a constant background noise (65 dB) that continued throughout the remainder of the session, followed by 52 presentations of acoustic stimuli to measure acoustic startle. The 52 acoustic trials consisted of: twenty-two 40 ms presentations of a 120 dB broadband pulse, ten 20 ms presentations of each prepulse intensity (68, 71, 77 dB) 100 ms prior to a 40 ms presentation of a 120 dB broadband pulse, and no stimulus trials in which no acoustic pulse was delivered in order to assess general motor activation in the rats. All trial types were presented in a pseudorandom order for a total of 60 trials (22 pulse-alone trials, 30 prepulse + pulse trials and 8 no stimulus trials). Five of the pulse-alone trials (120 dB), which were not included in the calculation of PPI values, were presented at the

beginning of the session to achieve a relatively stable level of startle reactivity for the reminder of the session. Another five of the pulse-alone trials, which were also not included in the calculation of PPI values, were presented at the end of the test session, with the remaining twelve 120 dB trials presented in the middle of the session. An average of 15 s (ranging from 7 to 23 s) separated consecutive trials. The total duration of the session was approximately 20 min [18].

2.5. Data analysis

2.5.1. Startle and PPI

Two measures were calculated from these data for each animal. First, the amount of PPI was calculated as a percentage score for each prepulse + pulse trial type: % PPI = 100 − {[(startle response for prepulse + pulse trial)/(startle response for pulse-alone trial)] × 100}. Second, startle magnitude was calculated as the average response to all of the pulse-alone trials. PPI data were analyzed with two-factor analysis of variance (ANOVA) with treatment as between-subjects factor and trial type (prepulse intensity) as repeated measure (within-subjects factor). Startle magnitude data were analyzed with one-factor (treatment) ANOVA. *Post hoc* analyses were carried out using Tukey's test. The alpha level was set at 0.05.

3. Results

Histological analysis revealed that the tips of the cannulae were situated inside the central or cortical dorsal nuclei of the IC, as shown in Fig. 1. Not all sites of injection are represented because of several overlaps. The effects of local administration of apomorphine or haloperidol into the IC on PPI and startle magnitude are depicted in Fig. 2A and B, respectively. Two-way ANOVA revealed a significant main effect of treatment [$F(2,26) = 9.0$; $P < 0.01$]. *Post hoc* analysis showed that the PPI response was disrupted in apomorphine treated rats across all prepulse intensities compared to the vehicle and haloperidol-treated groups. There was no main effect of the factor of prepulse intensity [$F(2,52) = 2.30$; $P = 0.068$], and no treatment × prepulse intensity interaction was observed ($P > 0.05$). There was no difference between vehicle and haloperidol-treated groups ($P > 0.05$). One-way ANOVA of startle magnitude on pulse alone trials did not reveal a significant effect of treatment between apomorphine and haloperidol-treated groups compared to the vehicle [$F(2,28) = 1.16$; $P = 0.206$]. Since there was no difference between the vehicle groups they were grouped together.

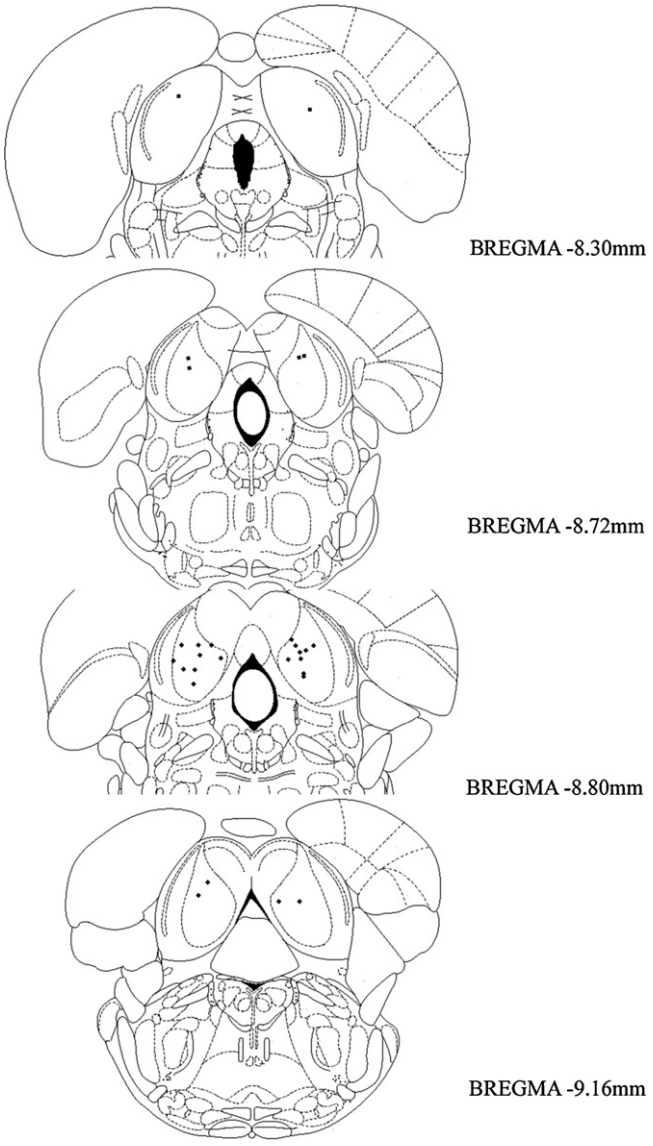

Fig. 1. Location of cannula placements (black dots) in the IC on cross-sections from the Paxinos and Watson [17] atlas. Figures represent the atlas coordinates in millimeters, posterior to bregma. Not all sites of microinjection are represented because of several dots overlaps.

4. Discussion

The present experiment tested the effects of intracollicular administration of apomorphine or haloperidol on PPI and the magnitude of the startle. The results demonstrate that apomorphine disrupted PPI at all three prepulses intensities, while haloperidol did not affect PPI suggesting that dopaminergic mechanisms in the IC may be at least partially responsible for the mediation of this effect. These findings are consistent with reports showing that stimulation, not blockade, of D2-family receptors by systemic administration of direct DA receptor agonists such as apomorphine substantially or completely disrupts PPI in rats [3,11,15,25]. In accordance with these results Melo et al. [16] showed that latent inhibition (LI), a model for the information filtering that underlies attentional processes was disrupted by apomorphine microinjection in the IC while microinjection of haloperidol into this structure did not interfere with LI.

Although it is well established that lesions of the IC disrupt PPI of acoustic startle [8,13,20] it was not previously known whether DA neurotransmission within the IC affect this response. The present study showed that apomorphine infused into the IC decreased PPI. Therefore, these results suggest that DA neurotransmission not only acts at PPI-modulating forebrain circuits (*e.g.* ventral tegmental area, nucleus accumbens, ventral pallidum, and pedunculopontine tegmental nucleus) [12] but also directly at lower level system, in the basic circuitry, mediating PPI.

In this study, the lack of a main effect of prepulse intensity was surprising. Typically we get more inhibition with increasing prepulse intensities. Nevertheless, despite the absence of a main effect of prepulse intensity, apomorphine was shown to disrupt PPI at all prepulse intensities.

An analysis of the startle response to the 22 pulse-alone presentations in our study did not reveal a significant main effect of treatment condition in the baseline startle magnitude supporting the contention that the startle reactivity and PPI have been shown to be independent measures. This is consistent with a large amount of evidence from other studies indicating that startle amplitude and PPI are independent phenomena and are highly dissociable [6,9].

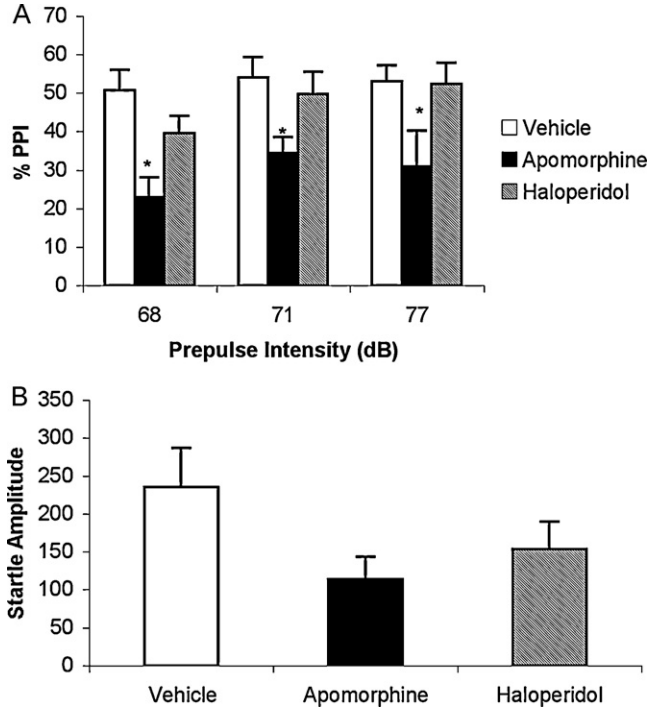

Fig. 2. Mean + S.E.M. percent of prepulse inhibition (A) and average startle amplitude (B) in males Wistar rats following bilateral microinjections of apomorphine (9.0 μg/0.5 μL) and haloperidol (0.5 μg/0.5 μL) into the inferior colliculus. $N = 11$ vehicle group; $N = 10$ apomorphine group; $N = 8$ haloperidol group.*$P < 0.01$ compared to vehicle and haloperidol.

Thus, deficits in PPI induced by apomorphine microinjection into the IC are not due to alterations in baseline startle reactivity.

In summary, the present findings suggests that dopaminergic neurotransmission of the IC can be involved in the mediation of PPI in rodents. Apomorphine disrupted PPI when microinjected directly to the IC. In contrast, microinjection of haloperidol into this structure did not seem to interfere with PPI.

These results suggest that activation or blockade of D2-family receptors of the IC differentially affects this response.

Contributors

Susan Satake and Karen Yamada are undergraduate students of the Universidade Federal de São Paulo. They have materially participated in the research, and were supported by FAPESP (proc.09/07347-0 and 09/07278-9). Dr. Melo, and Dr. Barbosa Silva were the professors in the Universidade Federal de São Paulo. They have participated in the article preparation. This work was sponsored by FAPESP (proc. 09/05703-4). All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report.

Acknowledgments

This work was sponsored by FAPESP (proc. 09/05703-4). S.Y. Satake and K.Y. Yamada were supported by FAPESP (proc. 09/07347-0 and 09/07278-9).

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