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Anxiolytic effects of intra-amygdaloid injection of the D1 antagonist SCH23390 in the rat

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Abstract

The intercalated islands are intra-amigdaloid clusters of D1 receptor rich GABAergic neurons, which control impulse traffic between the basolateral complex and the central nucleus of the amygdala. As dopaminergic transmission within the amygdala may play a role in anxiety, the effect of the D1 antagonist SCH23390 microinjected mainly close to the rostral intercalated islands in rats was studied, using the White and Black Box test. SCH23390 reduced anxiety by an increase in the latency of the first entry into the black compartment and by an increase in the total time spent in the white compartment of the White and Black Box test, while there was no significant modification of locomotion. It is suggested that blockade of D1 receptors in the rostral intercalated islands may reduce anxiety through a reduction of GABA-mediated dishinibition of the central amygdaloid nucleus.

Keywords: Anxiety; Amygdala; Black and White Box; Intercalated islands; Dopamine

Experimental evidence indicates that amygdala plays a key role in the integration and expression of fear and anxiety in several species of animals, including rats and humans [5,15]. Relevant sensory information from the environment is relayed to the lateral and basolateral amygdala from the cerebral cortex and thalamus. After processing, it is sent to the centromedial nuclear groups of the amygdala, through which an appropriate fear output is implemented. Projections from the medial and central amygdaloid nuclei to specific hypothalamic and midbrain targets give rise to different autonomic signs of fear and anxiety [5,15].

Convincing evidence by Paré and coworkers [22,23] has indicated that impulse traffic from the basolateral complex to the central nucleus of the amygdala is controlled by intercalated cell islands [16], clusters of inhibitory GABAergic cells [13,17] interposed between the basolateral complex and centromedial amygdaloid nuclei. The intercalated islands receive excitatory glutamatergic input from the basolateral nucleus and send inhibitory GABAergic fibers to the central nucleus [18,22,23]. It has been shown that these cell islands are rich in D1 dopamine receptor binding sites in comparison with other amygdaloid nuclei [24] and receive an important dopaminergic input [1,6]. This dopaminergic innervation of the intercalated islands has been shown to be preferentially directed to the rostrolateral main intercalated island and to the paracapsular intercalated islands, while there is only a sparse dopaminergic innervation of the rostromedial and caudal portions of the main intercalated island [6].

It has been reported that intra-amygdaloid injections of the specific D1 antagonist SCH23390 reduces the Fear-Potentiated Acoustic Startle response [12] and the Conditioned Freezing response in rats [7]. It is thus possible that dopaminergic mechanisms are involved in the modulation of

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fear output of the amygdala, through the activation of D1 receptors located on the intercalated cell islands. The aim of the present work is to further explore this possibility by injecting SCH23390 bilaterally in the vicinity of the rostral main intercalated and paracapsular islands which are innervated by DA terminals [1,6], and to measure the behavior of rats in an unconditioned test of anxiety, the White and Black Box test [4,9].

Male Wistar rats ($n = 38$) from 250 to 270 g (from Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Mexico City, Mexico) were housed individually in a controlled environment (temperature 22 °C, lights on 06:00–18:00 h) with water and food ad libitum. The experiments were conducted according to the guidelines established by the local Mexican Ethics Committee, and efforts were taken to minimize the suffering of the animals throughout all experimental procedures.

For implantation of permanent cannulas into the amygdala, the rats were anesthetized with ketamine hydrochloride (170 mg/kg, i.p.) and placed in a stereotaxic frame (Kopf Instruments, Tujunga, USA) with the incisor bar set at −3.3 mm. The body temperature was maintained at 37 °C using a CMA/150 Temperature Controller (CMA/Microdialysis, Stockholm, Sweden). Bilateral stainless steel guide cannulas 0.46 mm o.d., (C315G, Plastics One, Roanoke, USA) were positioned near the rostrolateral main intercalated island, (coordinates AP: −2.12 mm, L: ±4.7 mm, V: −7.0 mm) from bregma according to the atlas of Paxinos and Watson [19]. Guide cannulas were affixed with stainless steel screws and dental acrylic cement (Laboratorios Arias, Mexico City, Mexico) and sealed with a dummy cannula (C315DC, Plastics One, Roanoke, USA). Estreptobenzetacil V-Fortificado (20,0000 i.u., i.m.) was given to prevent infection (Fort Dodge Animal Health Labs, Mexico City, Mexico).

After 7 days of recovery, the rats were handled once a day for 5 min for 3 days. Rats were used for only one behavioral trial. In order to keep the animals in a similar alimentary condition and in this way to uniform at least to some degree their basal levels of anxiety the rats were kept since the night before the experiment in the experimental room without food but with water ad libitum. On the day of the experiment, either the D1 antagonist SCH23390 in saline or saline vehicle was injected bilaterally via an injection cannula, 0.20 mm o.d. (C315I, Plastics One, Roanoke, USA) which protruded 1 mm beyond the end of the guide cannula. Low doses (30 or 120 ng) of SCH23390 (Tocris, Ellisville, USA) in 0.2 μl saline or saline alone was microinjected bilaterally (0.2 μl/side) during a period of 5 min, using a CMA/100 Microinjection Pump (CMA/Microdialysis, Stockholm, Sweden). The cannulas were kept in place for 30 s after the injection to prevent backflow of the substance, and behavioral tests started immediately after the injection.

Behavioral experiments were carried out in a sound-attenuated room equipped with video-recording facilities. The boxes used for the evaluation of behavior were placed beneath the video camera, and the behavior was recorded in the absence of any observer. Anxiety-related behavior was measured using a White and Black Box test [4,9]. The box was made of acrylic and divided into two compartments, connected by a small opening (7 cm × 7 cm). The walls and floor of one compartment (27 cm × 27 cm × 27 cm) were in white, while those of a smaller compartment (27 cm × 18 cm × 27 cm) were in black. Squares of 9 cm × 9 cm were marked on the floor of both compartments using either black or white lines. During the experiments, the white compartment was strongly illuminated with a cold white light source (700 lx), whereas the black compartment was illuminated with a red light lamp (70 lx). At the beginning of the experiment, the rat was placed in the center of the white compartment facing the opening between the two compartments, and the behavior was video-recorded for 5 min. The latency of the first entry into the black compartment and the total time spent in the white compartment were taken as measures of anxiety [4,9]. The number of lines crossed in each compartment was recorded as an index of locomotion [4,9].

In addition, immediately after testing the animals in the White and Black Box test, locomotion was also measured in an Open Field (40 cm × 40 cm × 30 cm) divided in 16 squares (10 cm × 10 cm) as previously described [10]. The rat was placed in one of the corners of the box and allowed to explore the arena for 5 min. The number of square crossings was counted from the video-recordings, and these were determined as the number of instances that at least (3/4) part of the rat body reached the adjacent square. All experiments were conducted between 11:00 and 16:00 h. The rats were assigned to each group in a randomized way, which allowed to have rats injected with the different treatments at different times of the experiment.

At the end of the experiment, the animals were deeply anesthetized with sodium pentobarbital (65 mg/rat; Laboratorios Ttokkyo, Mexico City, Mexico) and 0.2 μl of a diluted solution of Pontamine sky blue (Sigma Chemical Co., St Louis, USA) was microinjected bilaterally via their injection cannulas. The rats were perfused intracardially with saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.6. The brains were removed and postfixed in the same fixative for 1–2 weeks. The placement of the cannulas was verified on coronal sections (45 μm) made with a cryostat (CM 1510-3, Leica Instruments, Nussloch, Germany) and counterstained with cresyl violet. Only rats with at least one cannula tip in the area of interest were included in this study ($n = 38$). In the White and Black Box test, the latency (the time to first enter the black compartment), and the total time spent in the white compartment are given in seconds and shown as medians with interquartile range. For the locomotion results the number of lines crossed in each compartment of the White and Black Box and the number of squares crossed in the Open Field test is presented as mean ± S.E.M. to facilitate the reading of Table 1 Since with a limited number of observations it is difficult to know whether the population under study