Activation of the Noergic System of the Nucleus Accumbens on Presentation of Contextual Danger Signals

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Contexts in which bright, emotionally colored events occur can subsequently operate as trigger stimuli initiating emotional reactions, becoming the decisive factor in selecting behavioral programs [4, 12]. An experimental model for this type of emotional memory is provided by conditioned reflex fear reactions to contextual stimuli arising on presentation of the chamber in which animals have previously been subjected to unavoidable electrocutaneous stimulation [5]. This conditioned reflex reaction often forms concurrently with the acquisition of conditioned reflex fear reactions to “classical” conditioned stimuli (tone + electric shocks delivered via the floor) and can contribute to the neurochemical and behavioral measures of the latter [11]. Published data provide evidence that the nucleus accumbens is among the brain structures involved in the formation and execution of conditioned reflex fear reactions to classical and contextual stimuli [5, 9, 10, 14], this being an area of the ventral striatum with an important role in controlling motivational and emotional processes of different modalities [11]. In particular, we have recently demonstrated that presentation of animals in a conditioned reflex chamber with a tone previously combined with electric shocks in this same chamber leads to increases in extracellular citrulline (a co-product of NO synthesis) in the medial segment of the nucleus accumbens. This increase was prevented by local administration of the NO synthase inhibitor 7-nitroindazole (0.5 mM). The increase was significantly smaller in amplitude than the increase in the citrulline level induced by combined presentation of the tone and the chamber but was no different from changes in citrulline levels seen during this test in the lateral segment of the nucleus accumbens. These data provide evidence that contextual danger signals activate neuronal NO synthase in the medial and perhaps the lateral segments of the nucleus accumbens, leading to increases in extracellular citrulline and, probably, increased NO production in this part of the brain.

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ronal NO synthase in this part of the brain. A further aim was to study the specificity of these changes in citrulline levels for the medial segment of the nucleus accumbens. This was addressed by determining whether extracellular citrulline levels in the lateral segment of the nucleus accumbens change during the execution of the conditioned reflex fear reaction to contextual stimuli, as this part of the nucleus accumbens is indicated by published data [9, 10] to be activated during this type of conditioned reflex behavior.

METHODS

Studies were performed using 52 male Sprague–Dawley rats weighing 260–330 g. Anesthetized (Rometar, 1.4 μg/100 g, and Zoletil, 5 mg/100 g, i.m.) animals underwent implantation of dialysis catheters into the medial (n = 44) and lateral (n = 8) segments of the right nucleus accumbens, as described previously [1–3, 15]. Microdialysis experiments were performed on the second post-implantation day. At the beginning of the experiments, rats were placed in daytime home cages and dialysis perfusion of the nucleus accumbens with artificial cerebrospinal fluid was started [15]. Animals with dialysis cannulae in the medial segment of the nucleus accumbens were divided into three experimental groups (experimental group 1, n = 15; experimental group 2, n = 8; experimental group 3 (n = 6) and two control groups (control group 1, n = 7; control group 2, n = 8). Rats with cannulae in the lateral segment of the nucleus accumbens constituted a further experimental group (experimental group 4, n = 8). A conditioned reflex fear reaction was developed in animals of all experimental groups 90 min after the beginning of the experiment, as described previously [2, 3]. Each rat was placed in the conditioned reflex chamber for 5 min, where it was presented with the conditioned stimulus (a tone of 1000 Hz for 10 sec) combined during the last second with electrocutaneous stimulation of the paw (0.5 mA, 1 sec). The rat was then returned to the daytime home cage. Training sessions were repeated after an interval of 1 h. The same procedure was followed with animals of control group 1, but without pain stimulation. Rats of control group 2 were placed in the conditioned reflex chamber for 5 min on two occasions, separated by 1 h, where they were presented with the same sound and pain stimuli (each stimulus was presented five times) non-simultaneously and in random order. During this control test, the only conditioned signal associated with the shocks was the chamber, as the sound, although presented, never coincided with the shocks. Baseline portions of dialysate (six samples, each of 5 min) were collected from animals of all groups after 25 min. Rats of experimental groups 1 and 4 and control groups 1 and 2 were then placed in the conditioned reflex chamber for 10 min, after which they were returned to their daytime home cages. After collection of baseline portions of dialysate, animals of experimental group 2 were also placed in the conditioned reflex chamber for 10 min, where they were presented with a tone (1000 Hz, 10 sec) each minute. Animals were then returned to their daytime home cages. In animals of experimental group 3, the perfusion solution was changed to solution containing a neuronal NO synthase inhibitor (7-nitroindazole, 7-NI, 0.5 mM, MB Biochemicals, USA) in artificial cerebrospinal fluid 25 min after dialysate collection started. After a further 30 min, they were placed in the conditioned reflex chamber for 10 min and then returned to their daytime home cages. Dialysate collection (5-min portions) in all groups was completed 20 min after return of rats to their daytime home cages. Citrulline levels were measured by HPLC with electrochemical detection [1]. A chromatography system as described previously [15] was used. Dialysate citrulline contents were expressed as percentages of individual mean pre-test values. Morphological monitoring of cannula positioning in the nucleus accumbens was performed when experiments were complete. Rats with cannulae located in the medial and lateral segments of the nucleus accumbens (depending on group) were included in the analysis (Fig. 1). Statistical analysis was performed using SigmaStat (3.0). changes in citrulline levels during behavioral tests and pharmacological treatments were compared with baseline values by unifactorial ANOVA. If this analysis revealed significant changes it was followed by comparison of changes at each time point with baseline levels using Student’s t test. Intergroup comparisons were performed by two-factor analysis of variance followed by comparison of groups at each time point using Student’s t test.

RESULTS

Baseline dialysate citrulline levels in the medial and lateral segment of the nucleus accumbens in these experiments were 31 ± 4 nM (n = 44) and 30 ± 7 nM (n = 8), respectively.

Presentation of animals of experimental group 1 (n = 15) with the chamber in which they had previously acquired the conditioned reflex fear reaction (combination of tone with shocks) led to a small but long-lasting (20 min) increase in the extracellular citrulline level in the medial segment of the nucleus accumbens compared with individual baseline values (Fig. 2, A; $F_{(1,154)}(11,66) = 9.2, p < 0.001$) with a peak (131 ± 6%; $t = 10.7, p < 0.001$) after the animals were returned to their home cages. This increase was also significant, as demonstrated by two-factor analysis of variance, as compared with the extracellular citrulline level in the medial segment of the nucleus accumbens of animals of control group 1 (n = 7) on presentation of the chamber without shocks (Fig. 2, A; $F_{(1,240)}(11,240) = 2.7, p = 0.003$). During this test, the citrulline level in animals of control group 1 showed no significant change from the individual pre-test baseline value (Fig. 2, A; $F_{(1,66)}(11,66) = 1.1, p = 0.4$).