Effect of Blocking of Glutamatergic Inputs to the Striatum on Regulation of Extracellular Dopamine Level in the Striatum by the *N. accumbens*

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INTRODUCTION

We have shown earlier [1, 2] that the *nucleus accumbens* exerts tonic effects on the synaptic release of dopamine (DA) and its metabolism in the dorsal striatum of rats and that the direction of the effects is determined by interaction between DA-ergic and glutamatergic afferents from the *n. accumbens* [1]. As known, the dopamine metabolism and release in the striatum can be modified either by a change in activity of DA-ergic neurons in the substantia nigra or by presynaptic interactions in the striatum proper. The two mechanisms may be involved in interaction between the *n. accumbens* and striatum, the former mechanism can act via projections of GABA-ergic neurons of the *n. accumbens* to cells of the medial region of the substantia nigra, which, in their turn, innervate the striatum. We have analyzed this pathway earlier [2, 3]. The second possible pathway of transmission of the *n. accumbens* effects, based on the presynaptic mechanism of DA-ergic functional regulation in the striatum, includes the following links: output neurons of the *n. accumbens* — ventral pallidum — medial thalamus — anterior cingulate cortex — dorsal striatum [4]. The neurotransmitter in corticostriatal projections is glutamate. In view of this, the main objective of our study was to explore a possible participation of the striatal glutamatergic inputs in the transmission of effects of the *n. accumbens* on striatal dopaminergic system. In this study we using the intrabrain dialysis in vivo explored the effect of blocking of the striatal glutamatergic inputs on shifts in extracellular DA concentrations in this structure, induced by application of DA-ergic substances to the *n. accumbens*.

METHODS

Experiments were conducted on 73 albino rats of the Sprague-Dawley line, weighing 250 to 300 g. Spiral-shape cannulae for dialysis were implanted into the striatum and *n. accumbens* of animals under a hexenal anesthesia [2]. The efficiency of such cannulae
for DA was shown in *in vitro* experiments to be of about 75%, i.e., the DA concentration at the cannula output amounted to 25% of the DA concentration in the perfused solution under study. The coordinates of cannula implantations were as follows: in the striatum, 0.5 mm dorsally from bregma, 2.5 mm laterally from the sagittal suture, at an insertion depth of 6.0 mm from the skull surface; in the *n. accumbens*, 2.5 mm rostrally from bregma, 1.2 mm laterally from the sagittal suture, at an insertion depth of 8.0 mm from the skull surface (Figure 1).

**Fig. 1.** Arrangement of dialysis cannulae. Cannulae (1, 2) were implanted into the *n. accumbens* (3) and dorsal striatum (4). The anterior commissure (5) is also indicated. Brain structures are depicted according to the atlas [15].

Dialysis experiments were performed 24 h after the implantation surgery, by the procedure described earlier [2]. Each rat was placed in the experimental chamber, where the striatum and *n. accumbens* were perfused with an artificial spinal fluid (ASF) at a rate of 2 μl/min.

After collection of the background dialyzate samples, the animals were divided into 10 groups. For three groups of rats the following blockers of excitatory amino acids were added respectively (in a concentration of 0.1 mM) to the ASF perfused through the striatum: kinurenic acid (KIN); diethyl glutamate (DG); and D,L-2-amino-5-phosphonovaleric acid (APVA) (Sigma, USA). Two groups of rats were perfused through the *n. accumbens* with addition of 1.0 mM of phenamine (standard pharmacopeic preparation, Russia) or haloperidol (Sigma, USA) in the ASF. Four groups of animals were treated by 1.0 mM haloperidol or phenamine, applied to the *n. accumbens* with striatal glutamatergic receptors blocked by the above-listed excitatory amino acid blockers (0.1 mM). For the tenth (control) group the composition of the ASF perfused through the two brain structures was unchanged during the experiment. The posttreatment striatum dialyzate was collected during 60 min. The dialyzate samples were stabilized [2], frozen (−20°C), and preserved till their analysis.

Each rat was used only once in the experiment. The DA level in the dialyzate was measured by the radioenzymatic technique (Catechola, Czechoslovakia) [5]. Methylated catecholamine derivatives were separated by thin-layer chromatography on Silufol plates (UV 254, Czechoslovakia). The radioactivity of samples was measured by a scintillation counter. The sensitivity of the method was of 0.02 pmoles. Changes in the DA concentration for each animal subject to the pharmacological actions were expressed in percentage units (the background level was taken as 100%). The Student's *t*-test was used for statistical processing of results.

**RESULTS**

The mean DA content, which in background dialyzate samples was of 0.21 ± 0.03 pmoles/50 μl, did not change in the course of the experiment, as demonstrated by the control group of rats (Table 1). The DA content in the dorsal striatum's extracellular space, with account of the efficiency of dialysis cannulae used, was of 16.8 nM. Phenamine application to the *n. accumbens* resulted in a 50% decrease in the extracellular DA content in the striatum. In contrast, addition of haloperidol to the solution perfused through the *n. accumbens* increased the DA level in the striatal extracellular space. Blocking of striatal glutamatergic receptors by DG or KIN, excitatory amino acid receptor blockers with a wide spectrum of action, increased, whereas application of APVA, a N-methyl-D-aspartate (NMDA) blocker, reduced the DA level in the striatum dialyzate. The dialytic perfusion of the *n. accumbens* with haloperidol against the background of KIN application to the striatum was accompanied by an increase in extracellular DA concentrations in the striatum by 240% on the average with respect to the background; this was significantly (*P* < 0.05) over the increase in the DA background level in the striatum dialyzate, caused by the KIN addition to the perfusing medium (177% with respect to the background). Haloperidol application to the *n. accumbens*, against the background of striatal NMDA receptor blocking by APVA, increased the DA content in the striatal extracellular space over that in background samples (Table 1) and after APVA application to the striatum (*P* < 0.001). Haloperidol, when applied to the *n. accumbens* concurrently with the striatum perfusion by a DG containing solution, induced practically the same increase in the DA level in the