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PHYSIOLOGY

Blockers of Monoamine Transporters Influence High Dopamine Concentration Uptake in Rat Brain Slices

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It is well known that the background extracellular concentration of dopamine in the brain is below 0.1 μM and only transiently increases to values of 1 μM after activation of dopaminergic neurons [1]. The high-affinity Na-dependent dopamine transporter (DAT) and norepinephrine transporter (NET) are involved in the maintenance of the extracellular level of dopamine in the brain. However, a number of drugs (cocaine, D-amphetamine, etc.) that block these dopamine transporters considerably increase the dopamine concentration, which determines their narcotic effect [2]. For example, it was shown that injection of cocaine induces a 14- to 17-fold increase in the average extracellular level of dopamine [3].

In addition, there are less specific monoamine transporters with an intermediate or low affinity to dopamine, which, however, have a considerable transport capacity (higher by a factor of 10–100), such as OCT/EMT (organic cation transporter/extraneuronal monoamine transporter) and PMAT (plasma membrane monoamine transporter) [4, 5]. These transporters are Na-independent and cocaine-insensitive.

Thus, dopamine is removed from the extracellular space by two mechanisms: high-affinity transporters that provide rapid uptake of small doses of the neurotransmitter (uptake-1) and low-affinity transporters that uptake high doses of the substance (uptake-2) [6]. It was shown that the anti-malaria drug quinine is a blocker of OCT transporters [7, 8]. Decynium-22 (or 1,1'-diethyl-2,2'-cyanine iodide) inhibits low-affinity monoamine transporters even stronger than quinine and does not affect the high-affinity transporters DAT and NET [9].

In this work, we blocked different stages of monoamine uptake with cocaine, quinine, and decynium-22 during the uptake of high (up to 40 μM) concentrations of dopamine by rat brain slices containing the frontal cortex, rostral area of the hippocampus, and midbrain to study role of low-affinity monoamine transporters.

The brain slices were prepared from Sprague-Dawley rats weighing 100–150 g. The brain tissue was cut at an angle of 30°, and the 350-μM slices contained part of the left and right frontal cortices and hippocampus, i.e., the structures with strong dopaminergic innervation (Fig. 1a). The level of dopamine was amperometrically measured using a Carbostar-4 carbon microelectrode with a resistance of 8 MΩ (Kation Scientific), a silver chloride electrode was used as a reference, and an Axopatch 1-D (Axon Instruments) in the two-electrode mode was used as a potentiostat. The electrode was placed in the solution close to the slice surface, and the working electrode had a potential of 370 mV necessary for dopamine oxidation. The current measured was filtered by a low-pass filter (10 Hz).

The changes in the dopamine concentration were measured in the following experiments: control-1, spontaneous oxidation of dopamine in the absence of reuptake (in a chamber not containing a slice); control-2, uptake of dopamine by untreated slices; experiment-1, uptake of dopamine by the slices treated with 15 μM cocaine; experiment-2, uptake of dopamine by slices treated with 100-μM quinine; experiment-3, uptake of dopamine by slices treated with 5 μM decynium-22.

All reagents were from Sigma (United States).

For statistical analysis, we used curves that illustrated a decrease in the dopamine concentration with time due to spontaneous oxidation and/or reuptake by the slice; the curves were approximated by an exponential equation using the formula $Y = A \cdot \exp(-K \cdot T)$, where Y is the rate of the decrease in the dopamine concentration in the incubation medium, which is equal to the rate of uptake by the slices, A and K are coefficients

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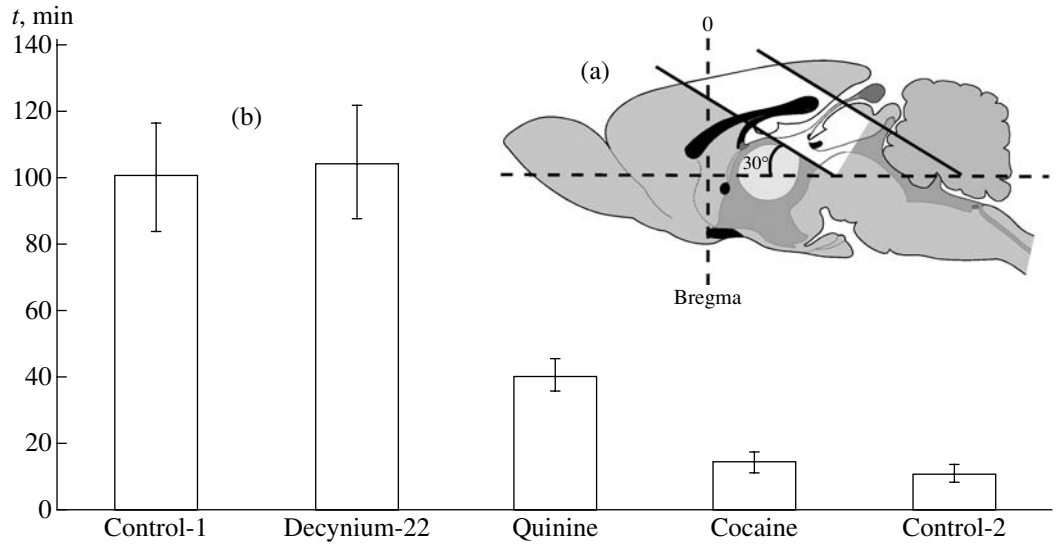

Fig. 1. (a) Orientation of the brain tissue block. (b) A diagram of dopamine uptake in control and experimental series. t is the time constant; $p < 0.05$.

t, min	100.1 ± 16.5	104.4 ± 17.2	40.7 ± 5.1	14.4 ± 3.1	10.8 ± 2.8
A	30.6 ± 2.1	29.0 ± 1.8	24.5 ± 3.8	28.9 ± 2.0	27.6 ± 1.9
K	0.007 ± 0.001	0.004 ± 0.0003	0.017 ± 0.003	0.048 ± 0.005	0.063 ± 0.004
Number of experiments	$n = 8$	$n = 6$	$n = 6$	$n = 6$	$n = 8$

that described the shape of the curve, and T is time. We determined the time constant (t is the half-life of the exponent), which described the process of dopamine uptake by the slice. The final concentration of dopamine was taken to be zero. All characteristics were averaged over experiments. The same procedure was used to measure the characteristics of dopamine uptake in the presence of inhibitors. Statistical comparison of the groups was performed with the use of Student's t test by means of the Grafpad Prism 4.03 software.

In our experiments, the time constant was the best value that characterized the rate of dopamine uptake by the slices (Fig. 1b). In control-1, in the presence of 40 μM dopamine (higher concentrations were toxic for the tissue), 3–4 min after stabilization, we observed a decrease in the concentration with a time constant of $t = 100 \pm 16$ min, which was the largest in our experiments. We believe that this is the time of dopamine spontaneous oxidation, because the carbon microelectrode at a current of 30 pA negligibly contributed to oxidation. The dopamine uptake by the brain slice in the absence of inhibitors (control-2) was characterized by $t = 10.8 \pm 2.8$ min, i.e., the uptake of dopamine in this case was much faster. Thus, control-1 is the slowest model of decrease in the dopamine concentration and control-2 is the fastest model.

In the next series of experiments, dopamine was supplemented with one of inhibitors of dopamine transporters: 15 μM cocaine (experiment-1), 100 μM quinine (experiment-2), or 5 μM decynium-22 (experiment-3). Figure 1b shows that addition of any of these agents inhibited the uptake of dopamine as compared to control-2. Cocaine increased the time of dopamine uptake by 40%, whereas quinine was three times more effective and increased the uptake time by a factor of 4. Decynium-22 almost completely inhibited the dopamine uptake by the slice, the time constant ($t = 104.4 \pm 17.2$) not differing significantly from the dopamine spontaneous oxidation time ($t = 100.1 \pm 16.5$, control-1). Thus, decynium-22 induced a 10-fold decrease in the rate of dopamine uptake as compared to an intact slice.

What is the role of low-affinity transporters in the uptake of high concentrations of dopamine by the slices?

After cocaine-induced inhibition of high-affinity transporters, the level of dopamine increased and depended on the activity of high-capacity, low-affinity transporters; this Na-independent transport of monoamines was found in glial cells [10]. This group of transporters functions at abnormally high concentrations of dopamine in the extracellular space and their inhibition, in turn, should additionally decrease the dopamine uptake.

In studies on the uptake of dopamine by the rat prefrontal cortex, the dopamine concentration was about 2 μM (point A in Fig. 2), which corresponds to the maximum activity of DAT and NET and uptake of almost all dopamine by these transporters [11]. OCT becomes saturated only at high concentrations of 200–4000 μM [4]. However, this high concentration of dopamine is toxic for the slice; hence, in our experiments, we used an ini-

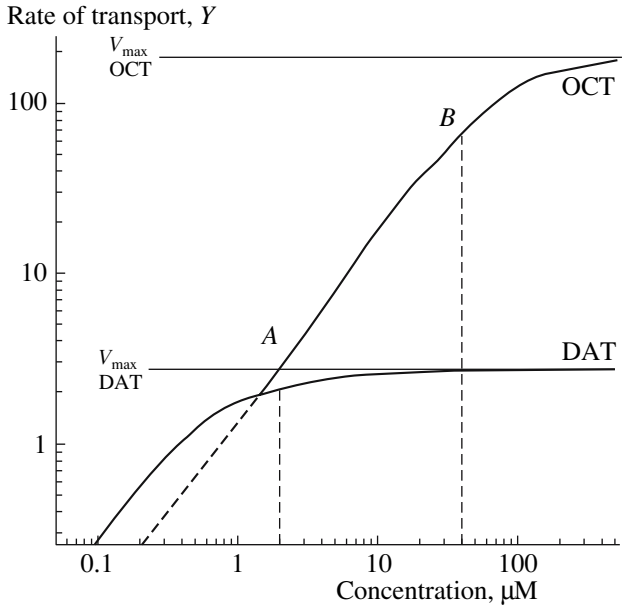

Fig. 2. Kinetics of OCT (the low-affinity dopamine transporter) and DAT (the high-affinity transporter). Concentration of dopamine: (*A*) 2 μM; (*B*) 40 μM.

tial concentration of dopamine of 40 μM (point *B* in Fig. 2).

Quinine is not an inhibitor of high-affinity dopamine transporters and, hence, does not have a narcotic effect; quinine does not induce direct addiction, but it was shown that the preference to this substance is developed after treatment of rats with the drug [12]. Decynium-22 is a considerably stronger inhibitor, which blocks OCT and PMAT [5] and reduces the rate of dopamine uptake to the rate of spontaneous oxidation (Fig. 1b). The low-affinity transporters in astrocytes [5] are involved in work at elevated concentrations of dopamine, hence, the rate expression of these transporters may determine tolerance to the drug. Thus, understanding the dopamine uptake by low-affinity transporters may shed light on the mechanisms of formation of drug abuse.

It is also known that levodopa, a metabolic precursor of dopamine, is used for replacement therapy of Parkinson's disease [13]. Prolonged treatment of patients with levodopa results in habituation and necessity to increase the dose, which induces side effects: hyperkinesia, choreoathetosis, depression [14, 15], and symptoms of schizophrenia [6]. It is possible to hypothesize that the important component of habituation to the action of levodopa is compensatory enhancement of the activity of systems responsible for uptake of a high dopamine concentration, including low-affinity monoamine transporters. Presumably, reversible inhibition of these excessively active transporters, which was shown in our experiments, will help to decrease therapeutic doses of levodopa and considerably prolong the effect of single treatment of this compound in patients with Parkinsonism.

Cocaine (15 μM) did not affect the pattern of uptake of high concentrations of dopamine (40 μM). Quinine (100 μM) and decynium-22 (5 μM) efficiently blocked the uptake of high concentrations of dopamine, and decynium-22 almost completely reduced the rate of dopamine uptake to the rate of spontaneous oxidation.

This ensures the involvement of low-affinity dopamine transporters (which may be blocked by quinine and decynium-22) in the removal of high concentrations of dopamine in the brain.

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