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PRESYNAPTIC CONTROL OF DOPAMINE RELEASE BY β-PHENYLETHYLAMINE

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It has been suggested on the basis of existing experimental data that endogenous [7, 11] β-phenylethylamine (β-PEA) can modulate the efficiency of dopaminergic synaptic transmission [4, 6, 15] through its action on the presynaptic [4, 8] and postsynaptic [3] parts of the dopaminergic synapse. The presynaptic action of β-PEA has been shown to include both potentiation (facilitation) of dopamine (DA) release and, although admittedly to a much lesser degree, inhibition of DA reuptake by dopaminergic endings [4].

It was accordingly decided to study the effect of extracellular ions (Ca^{++}, Na^{++}) on the β-PEA releasing effect, dependence of this effect on the membrane potential of dopaminergic endings, and the participation of dopamine presynaptic autoreceptors in the realization of the effects of β-PEA on DA release.

EXPERIMENTAL METHOD

The method of local perfusion of the brain by a push-pull cannula system, described by the writers previously, with simultaneous recording of brain electrical activity from the region of perfusion [2] was used. Experiments were carried out on noninbred male albino rats weighing 300-350 g, anesthetized with pentobarbital (40 mg/kg). During the preliminary operation a guide tube with the recording electrode (AP = −1.5; L = 2.5; V = 5) was inserted into the neostriatum and stimulating electrodes (AP = 4.5; L = 2; V = 8.5) were inserted into the substantia nigra stereotaxically, using the atlas of Fifkova and Marsala. The parameters of stimulation were threshold strength for evoked potential generation in the region of the neostriatum (30 V, 0.1 msec). By means of a microsyringe, [^{3}H]-DA hydrochloride ([^{3}H]-DA) in a dose of 3-4 μl, $1.25 \cdot 10^{-9}$ M (specific activity 3.2 Ci/mmole, from Amersham Corporation, England) was injected through the guide tube into the region destined for perfusion. After a 30-min period of application necessary for uptake of the exogenous DA by the cells, perfusion of that region of the brain began. The push-pull cannula was inserted into the neostriatum through the guide tube as far as the level of the tip of the recording electrode. An artificial CSF of the following composition was used for perfusion (in mM): NaCl 126.5, KCl 2.4, $CaCl_2$ 1.1, KH_2PO_4 0.5, $NaHCO_3$ 27.5, $MgSO_4$ 1.1, glucose 5.9 [12], with the addition of the monoamine oxidase inhibitor iproniazid (10^{-6} M). Before the experiment the perfusion fluid was

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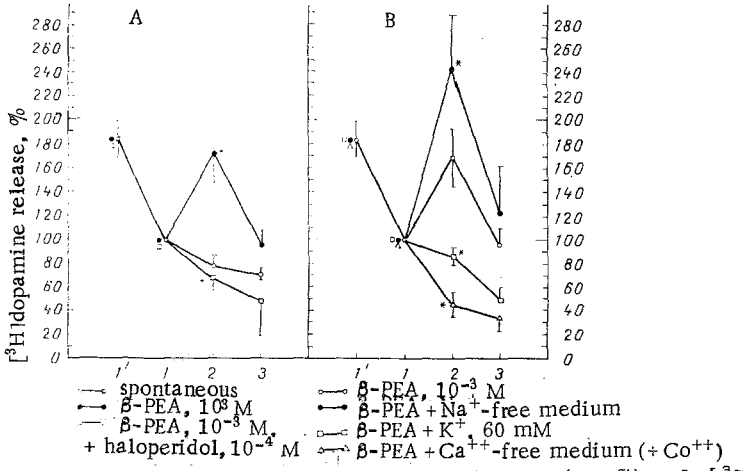

Fig. 1. Effect of β-PEA on spontaneous release (in %) of [^{3}H]-DA into perfusate. 1, 2, 3) 20-min fractions of perfusate collected 60 min after beginning of perfusion of rat neostriatum. 1') 20-min fraction of perfusate preceding first fraction. In all experiments composition of perfusion medium was changed during second 20-min perfusion period. Asterisks indicate significantly different mean values of [^{3}H]-DA release in analogous periods of perfusion in two groups of experiments compared (3-4 experiments, M ± m); *P < 0.05; **P < 0.01.

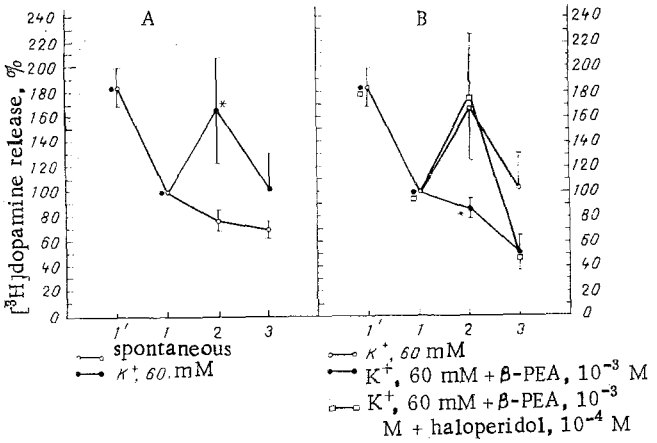

Fig. 2. Effect of β-PEA on K^+-induced release (in %) of [^{3}H]-DA into perfusate. Legend as to Fig. 1.

saturated with carbogen (95 O_2 + 5% CO_2) to pH 7.4. The rate of flow of the perfusion fluid was 50 µl/min and the total duration of perfusion 2-3 h. The perfusion fluid collected during the first 60 min after the beginning of perfusion was not analyzed, for during this time exogenous ^{3}H -DA not bound with cells is washed out. The three subsequent 20-min fractions of perfusate were analyzed. They were collected in cold measuring tubes containing 100 µl of solution of the following composition: 0.2% EDTA, 6% ascorbic acid, 0.005% DA [5, 9]. [^{3}H]-DA was isolated from these fractions on columns (4 × 120 mm) filled with ion-exchange resins Dowex 50W × 10, 20/50 mesh (h = 50 mm) and Dowex 50W × 4, 100/200 mesh (h = 70 mm) (from Serva, West Germany) in the Na^+ form by Volina's method [1] with certain modifications. Aliquots (0.6-0.8 ml) of fractions of perfusate, neutralized beforehand with 3 N Na_2CO_3 solution to pH 6.0-6.5, were applied to the column. The rate of flow of the fluids through the column was 0.7 ml/min and was kept constant by means of a water-jet vacuum pump. The tubes containing the perfusate were washed twice with deionized water (5 ml each time), which was passed through the corresponding columns. The [^{3}H]-DA was eluted with 10 ml of 2N HCl. The eluates were collected in counting flasks and dried at 90°C. The radioactivity of the dried eluates was estimated on an Sh-4000 scintillation counter (Intertechnique, France) after the addition of toluene scintillator. Radioactivity of the HCl eluate from the column after application of the first 20-min fraction of perfusate was taken as 100% and release of [^{3}H]-DA during the

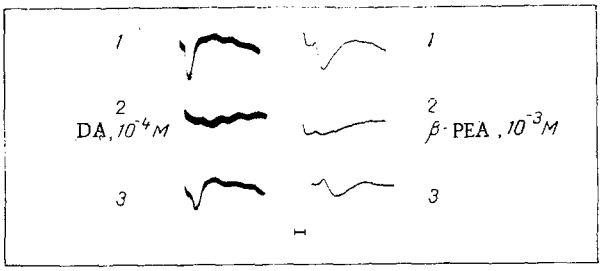

Fig. 3. Effects of β-PEA and DA on evoked potentials recorded in region of perfusion of neostriatum during electrical stimulation of compact zone of rat substantia nigra. Evoked potentials averaged for 10 realizations. 1) in first 20-min period 60 min after beginning of perfusion, 2) on addition of DA (10^{-4} M) or β-PEA (10^{-3} M) to perfusion medium in second 20-min period of perfusion, 3) in third 20-min period of washing with normal perfusion medium. Time marker 5 msec.

next 20-min periods of perfusion was estimated relative to it. The composition of the perfusion medium was changed during the second 20-min period. In the third 20-min period the sample was washed with normal perfusion medium. The significance of the difference in levels of [^{3}H]-DA release during analogous periods of perfusion in the groups of animals compared was estimated by Student's test. At the end of the experiment the position of the cannula and electrodes was verified morphologically.

EXPERIMENTAL RESULTS

Experiments *in vivo* with local perfusion of the rat neostriatum showed that β-PEA, if added to the perfusion medium in a concentration of 10^{-3} M, increases (up to 172 ± 25.1%) release of [^{3}H]-DA, applied beforehand to the neostriatum, into the perfusate (Fig. 1, I). This effect was reduced (to 56 ± 11.7%) in calcium-free perfusion medium (in the presence of an equimolar concentration of Co^{++} ions), which suggests a role for Ca^{++}-dependent mechanisms in the effect of β-PEA (Fig. 1, II). Release of [^{3}H]-DA into the perfusate induced by β-PEA was not observed in the presence of haloperidol (10^{-4} M) in the perfusion medium (68 ± 8.2%; Fig. 1, I). This action of haloperidol is perhaps linked with its possible inhibitory effect on penetration of β-PEA into dopaminergic nerve endings, for we know that haloperidol, in these concentrations, can inhibit DA reuptake [10]. Release of [^{3}H]-DA into the perfusate rose sharply if β-PEA (10^{-3} M) was added to sodium-free perfusion medium, in which NaCl was replaced by choline chloride in an equimolar concentration (Fig. 1, II). The reason for this, in the existing view [13], is probably two processes that are observed on reversal of the Na^+ gradient, namely inhibition of [^{3}H]-DA reuptake and potentiation of [^{3}H]-DA release mediated by the membrane carrier. By contrast with the stimulating effect on spontaneous release of [^{3}H]-DA into the perfusate, β-PEA (10^{-3} M) inhibited (up to 87 ± 6.7%) [^{3}H]-DA release evoked by K^+-depolarization (Fig. 2). Since β-PEA can activate postsynaptic dopamine receptors in the rat neostriatum [3], it was suggested that the inhibitory effect of β-PEA on K^+-induced [^{3}H]-DA release was connected with the action of β-PEA on presynaptic dopamine autoreceptors. To test this hypothesis experiments were carried out with haloperidol, a blocker of dopamine receptors. Haloperidol (10^{-4} M) was found to abolish the inhibitory effect of β-PEA on K^+-induced [^{3}H]-DA release and to restore release to the K^+-induced level (to 174 ± 52%; Fig. 2, II). Further evidence in support of the inhibitory action of β-PEA on stimulus-induced DA release is given by the fact that on the addition of β-PEA (as of DA itself) to the perfusion medium a decrease in amplitude of evoked potentials recorded in the region of perfusion of the neostriatum was observed during electrical stimulation of the substantia nigra, suggesting a decrease in DA release from the nerve endings in response to the arriving action potential (Fig. 3).

These experiments *in vivo* thus demonstrated the ability of β-PEA to regulate DA release in different directions depending on the functional state of the dopaminergic neuron. With normal (or depressed) functional activity of the dopaminergic neurons β-PEA evidently induces a releasing effect because of penetration into dopaminergic endings [14]. If, however, the presynaptic membrane of the dopaminergic ending is depolarized, β-PEA, without penetrating

inside the terminal, probably activates presynaptic dopamine autoreceptors, reducing stimulus-induced DA release.

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FORMATION OF ADDICTION TO NICOTINE IN NONINBRED ALBINO RATS

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KEY WORDS: nicotine; addiction; rats.

Tobacco smoking is currently the most widespread type of toxicomania, which is based on the pharmacologic effects of nicotine [3-6].

The search for drugs for the treatment of tobacco smoking, which has not yet proved very effective, is made more difficult by the absence of a pathogenetically based experimental model of this toxicomania. The aim of the present investigation was accordingly to study the possibility of formation of addiction to nicotine in laboratory rats and to create an experimental model of nicotine toxicomania on this basis.

EXPERIMENTAL METHOD

The formation of addiction to nicotine was studied on 56 male rats weighing 190 to 210 g. The rats were kept in individual cages measuring 40 × 12 × 15 cm, equipped with a feeding bowl (food was provided *ad lib.*) and with two graduated vessels containing water and 0.005% nicotine solution for a period which ranged from 2 months (26 rats) to 4 months (30 rats). The quantity of water and of nicotine solution drunk per day by each rat was recorded and preference of each animal for a particular liquid was calculated. The results were subjected to statistical analysis with calculation of the significance of differences between fractions [1].

EXPERIMENTAL RESULTS

Rats kept in individual cages with choice allowed between water and 0.005% nicotine solution could be divided after the first day into three groups on the basis of preference for one or other fluid (Table 1).

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