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LOCATION OF DOPAMINE RECEPTORS ON THE NERVE CELL MEMBRANE

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It is generally accepted that mediators of synaptic transmission interact with specific receptors located on the outer surface of nerve cells. Recently, however, evidence of the effectiveness of various mediators when introduced intracellularly has been published [5-7]. This has raised the urgent question of whether the effects of these mediators are the result of their penetration inside the neuron through the cell membrane.

To study this problem, it is convenient to use immobilized mediators, i.e., mediators chemically bound with a certain carrier substance which prevents their intracellular penetration. For this purpose we have used dopamine (DA), bound with a high-molecular-weight polymer. The choice of DA was determined by the similarity of its membrane effects (membrane depolarization) when applied to neurons extracellularly and intracellularly [4, 6]. DA bound with polymer (DA-P) and low-molecular-weight DA were applied to the outer surface of the membrane of sensomotor cortical neurons of a rabbit by microiontophoresis.

EXPERIMENTAL METHOD

Experiments were carried out on 11 adult rabbits weighing 3.0-4.0 kg, anesthetized with urethane (750 mg/kg) and chloralose (30 mg/kg). Altogether 49 sensomotor cortical neurons were tested.

Action potentials (AP) of the neurons were recorded extracellularly through one barrel of a seven-barreled glass microelectrode, filled with 3 M NaCl. The other barrels of the microelectrode were filled with aqueous solutions of the following substances: DA hydrochloride (0.14 M, pH 4.0), DA bound with polymer (0.14 M calculated as low-molecular-weight dopamine, pH 4.0), droperidol (0.02 M, pH 4.5), sodium glutamate (1.0 M, pH 7.3), and the carrier polymer (38%, pH 4.0). One barrel of the microelectrode was filled with 3 M NaCl as control for polarization effects of the current and to compensate current artifacts.

DA-P was purified twice by gel chromatography on Sephadex G-50. Absence of contamination with low-molecular-weight DA was verified by thin-layer chromatography on Silufol in a system of methanol—acetic acid (100:1). The DA content in DA-P, measured spectrophotometrically, was 7.0%.

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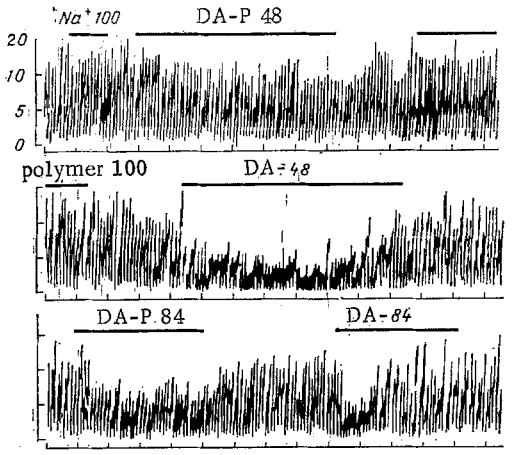

Fig. 1

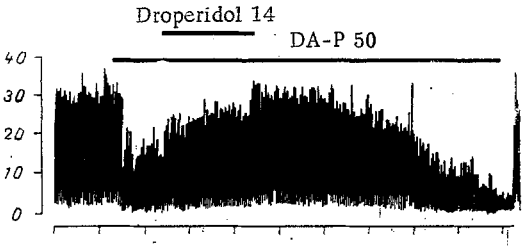

Fig. 2

Fig. 1. Inhibitory effect of DA bound with polymer (DA-P) and of low-molecular-weight DA on electrical activity of sensomotor cortical neuron. Abscissa, time (in min); ordinate, frequency of neuron (spikes/sec). Horizontal lines above traces indicate duration of microiontophoretic application of agents, numbers above them indicate strength of current (in nA). Each bottom trace is a continuation of the one above.

Fig. 2. Reduction of inhibitory effect of DA-P by droperidol. Spontaneous unit activity maintained at a high level by continuous microiontophoretic application of sodium glutamate by a current of 12 nA. Legend as in Fig. 1.

TABLE 1. Correlation between Different Types of Responses of Sensomotor Cortical Neurons to Microiontophoretically Applied DA-P and DA

DA	DA-P			
	+	−	no effect	total
+	3	0	1	4
−	0	34	1	35
No effect	2	0	8	10
Total	5	34	10	49

Legend. Numbers indicate number of neurons; +) increase in frequency of AP; −) decrease in frequency of AP.

EXPERIMENTAL RESULTS

As Table 1 shows, the main effect of DA-P, like that of DA not bound with polymer, was a decrease in the spontaneous discharge frequency of the cortical neurons (34 or 49 neurons). An excitatory effect of DA-P was found in only five neurons, and the electrical activity of 10 neurons was unchanged by DA-P. Under identical conditions of microiontophoretic application (equimolarity, equality of currents expelling the substances, etc.) the depressant effect of DA-P on unit activity was weaker and developed more slowly than the effect of low-molecular-weight DA.

Characteristics of typical effects of DA-P and DA, applied to the same neuron, are illustrated in Fig. 1. The inhibitory effect of DA-P, like the effect of DA, depended on the dose of the substance applied, and was potentiated by an increase in the microiontophoretic current from 48 to 84 nA. DA caused deeper depression of unit activity than DA-P. A stable inhibitory effect of DA-P (flattening out on a plateau) developed slowly — about 60 sec after

application of the microiontophoretic current. The effect of DA developed much faster — during the first 10-20 sec. Recovery of the initial level of unit activity after discontinuation of the microiontophoretic current also was delayed in the case of DA-P compared with DA. The absence of escape of the neuron from the inhibitory action of DA-P during prolonged application of the agent will be noted. Escape was distinctly observed during microiontophoretic application of DA. In control tests in which a strong microiontophoretic current was passed through 3 M NaCl and polymer not bound with DA, electrical activity of the neuron was unchanged.

The amplitude of AP of many neurons increased by 5-30% during microiontophoretic application of both DA-P and DA.

Droperidol, a pharmacologic blocker of dopamine receptors, reduced or completely abolished the inhibitory effects of DA-P in 6 of 8 neurons studied (Fig. 2).

It follows from these results that DA, when incorporated into the structure of a polymer, does not lose its ability to interact with specific receptors and to exert a depressant action on cortical neurons, like low-molecular-weight DA itself. Evidence of the specificity of interaction of DA-P with DA-receptors is given both by dependence of the strength of the effect of the dose of DA-P applied and the reduction of the effect of DA-P by the specific blocker of DA-receptors, droperidol.

The increase in amplitude of AP of the neurons under the influence of DA and DA-P observed in these experiments may be evidence of hyperpolarization of the neuron membrane. This contradicts the observations of Bernardi et al. [4], who found membrane depolarization of cortical neurons and a decrease in amplitude of their AP in an intracellular study under the influence of microiontophoretically applied DA.

The weaker inhibitory effect of DA-P than that of DA, and also the delayed development of the effect of DA-P after the beginning of its application to the neuron can evidently be attributed to the slower rate of electrophoretic transport of the DA-P molecules because of their greater molecular weight, and also of conformational screening of DA molecules by the polymer in the structure of DA-P. Slow recovery of the original unit activity after discontinuing microiontophoretic application of DA-P and absence of the phenomenon of escape of the neuron from the inhibitory effect of DA-P, characteristic of DA [1], can perhaps be explained by interference with the processes of inactivation of DA when bound with the polymer. The most important of these processes are enzymic release of DA and its reuptake by nerve endings.

For DA to exhibit its effect as mediator (or modulator) of unit activity, it therefore need not penetrate inside the neuron through the cell membrane. Specific receptors sensitive to DA are located on the outer surface of nerve cells, and activation of these receptors is not significantly prevented by incorporation of DA into the structure of a polymer, preventing penetration of the DA inside the cell.

Mineeva et al. [2], who studied regulation of the kinetic properties of tyrosine hydroxylase in brain synaptosomes, also found that the properties of the DA analog are similar to those of low-molecular-weight DA. In the model used by these workers, moreover, DA-P was actually more active as an agonist of DA-receptors than its low-molecular-weight analog.

Our findings also agree with those of Poskonova et al. [3], obtained in experiments with intracellular dialysis of neurons of the snail *Limnaea stagnalis*. Intracellular acetylcholine is found to diffuse through the cell membrane and to exert its specific membranotropic action only by interacting with receptors located on the outer side of the nerve cell membrane.

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