PENETRATION OF CATECHOLAMINES THROUGH THE BLOOD-BRAIN BARRIER

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The ability of intravenously injected catecholamines to penetrate through the blood-brain barrier was studied in experiments on albino rats and rabbits. It was shown by the method of Euler and Lishaiko that after intravenous injection of noradrenalin its concentration in the hypothalamus rises. After injection of tritium-labeled adrenalin and DL-3,4-dihydroxyphenylalanine (DOPA), radioactivity was found in both parts of the brain tested, although in a much smaller concentration in the cerebral cortex. The results of autoradiographic studies showed that the isotope (adrenalin-H^3, DOPA-H^3) penetrates both into the cytoplasm and into the nucleus of the neurons, and that most of it is found along the outer and inner borders of the nuclear membrane.

Penetration of catecholamines through the blood-brain barrier (BBB) has not yet been finally settled. Many workers deny that catecholamines can penetrate from the blood into the brain and they explain this by the high polarity of catecholamine molecules and their low solubility in lipids [5, 6]. However, results have recently been obtained to show that after intravenous injection of catecholamines it is possible for central effects to arise [4, 7, 8], similar to those observed when the noradrenalin precursor DL-3,4-dihydroxyphenylalanine (DOPA) is used [1]. In particular, Zakusov [3] has shown that noradrenalin and adrenalin, if injected intravenously in small doses, cause changes in the summation of impulses in the central nervous system similar to the phenomenon observed after their injection into the cerebral ventricles or after intravenous injection of DL-3,4-DOPA. Direct neurochemical experiments also have yielded results indicating the selective permeability of the BBB to catecholamines in the region of the hypothalamus [10].

It can be concluded from these facts that the observed effect is connected with the central action of the catecholamines. It was therefore interesting to determine to what extent catecholamines, if injected intravenously, pass through the BBB.

EXPERIMENTAL METHOD

The writers' modification [2] of Euler and Lishaiko's spectrofluorometric method of determination of noradrenalin was used in conjunction with radioactive indicator methods to investigate this problem. Experiments were carried out on male rabbits weighing 2.5-3 kg and male albino rats weighing 160-180 g. Fluorescence was recorded with a fluorometer (Opton). The degree of penetration of labeled catecholamines into the brain was determined by means of a scintillation counter (Nuclear Chicago) and by histoautoradiography. Adrenalin-H^3 (specific activity 8.8 Ci/g) and DL-3,4-DOPA-7,8-H^3 (specific activity 490 mCi/g) were used in the experiments. In all series of experiments the catecholamines were injected intravenously in doses (1-10 μg/kg) causing changes in the summation activity of the central nervous system. The animals were decapitated 2 min after injection of the catecholamines and the brain was removed. The cerebral cortex and hypothalamus were taken for investigation. Pieces of brain tissue were treated by

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Fig. 1. Autoradiographs of brain sections of intact rats. Hypothalamic region. Intravenous injection of adrenalin-H^3 (a) and DL-3,4-DOPA-H^3 (b). Hematoxylin, 900×.

TABLE 1. Penetrating of Labeled Catecholamines into Some Parts of the Brain

Animal	Substance	Dose (in μg/kg)	Activity (in μCi/kg)	Number of impulses per milligram brain tissue	
				cortex	hypothalamus
Rabbits	Adrenalin-H^3	1,2	10	144 (115—173)	275 (215—235)
Rats	The same	1,2	10	126 (119—133)	293 (226—350)
"	" "	6,0	50	1 408 (1 252—1 664)	1 996 (1 850—2 142)
"	DOPA-H^3	330,0	165	235 (201—269)	433 (381—585)

Petroff's method [9]. Radioactivity was counted as H^3 γ rays and expressed as the number of pulses per milligram tissue.

For the histoautoradiographic investigations the animals' brain was divided by 2 frontal incisions at the level of the optic chiasma and superior colliculi. The piece of tissue excised was placed for fixation in Carnoy's solution, and embedded in paraffin wax, and cut into sections 5-7 μ in thickness. The sections were then dewaxed, coated with type M emulsion, and exposed for 40 days. The autoradiographs were then stained with hematoxylin and fixed. During the analysis the number of grains of reduced silver above the corresponding area of brain tissue in five fields of vision (MBI-3 microscope with 1× 1.5 binocular attachment, ocular 10, objective 90, oil immersion) was counted in each autoradiograph and the mean calculated. Autoradiographs of identical areas of the brain from 5 rats were counted in this way. The background level was calculated from these results. If the quantity of label in a corresponding area of the section was more than twice the background value it was considered that radioactive indicator (adrenalin-H^3, DOPA-H^3) was present in that area.

EXPERIMENTAL RESULTS

In the experiments of series I changes in the noradrenalin level in the cortex and brain stem were studied after its intravenous injection. The noradrenalin concentration in the hypothalamic region was found to be increased, while in the cerebral cortex it remained unchanged. For instance, the noradrenalin concentration in the hypothalamus of the control animals was 59.1 (54.2-63.8) μg/kg, while after intravenous injection of noradrenalin its concentration rose to 89.2 (84.5-102.6) μg/kg.

To rule out any effects of endogenous release of catecholamines in the brain tissue on the results, experiments were carried out using labeled catecholamines (adrenalin-H^3, DOPA-H^3). These experiments showed that intravenously injected adrenalin-H^3 and DOPA-H^3 are found in both parts of the brain which were studied, although to a much lesser degree in the cortex (Table 1).

The results of the histoautoradiographic investigations of brain sections of rats receiving adrenalin-H^3 reflected the selective localization of the isotope in hypothalamic neurons. In the animals receiving DOPA-H^3 radioactivity was also found in the cerebral cortex. In all series of experiments tritium label was found both in the cytoplasm and in the nuclei of the nurons, and was concentrated mainly along the outer and inner borders of the nuclear membrane (Fig. 1).

The results of this series of experiments indicate that the BBB is permeable to catecholamines not only in the hypothalamus, but also in the region of the cerebral cortex. The well-defined localization in the hypothalamic neurons will be noted. It can accordingly be concluded from these results that changes in the summation power of the central nervous system following injection of the catecholamines are the result of their central action.

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