INTRACELLULAR MECHANISM OF ACTION OF IMIPRAMINE AND DIAZEPAM

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Subcellular fractionation of the tissue of the hypothalamus was carried out by differential centrifugation in experiments on rats. The effect of the antidepressant imipramine and the tranquilizer diazepam on the noradrenalin content was determined in the isolated fractions. The results showed that imipramine lowers the noradrenalin content in the synaptosomes but diazepam has no such effect. The adrenergic component thus plays an important role in the mechanism of action of the first of these substances, but not in the action of the second.

KEY WORDS: noradrenalin; hypothalamus; imipramine; diazepam.

The method of differential centrifugation of brain tissue, which has recently become very widely used in research, has opened up new prospects for the study of the mechanism of action of neurotropic drugs. Investigations on isolated nerve endings (synaptosomes) are most useful for the study of psychotropic agents whose action is known to be connected with their effect on synaptic transmission [3].

Previous investigations showed the effect of several psychopharmacological agents on the enzymes of mediator metabolism [2, 5]. To continue this line of research, it was important to investigate the content of noradrenalin (NA) in the synaptosomes under the influence of drugs with different types of action.

In the investigation described below the effect of the antidepressant imipramine and of the tranquilizer diazepam on the NA content in subcellular fractions isolated from the hypothalamic region of the brain was studied.

EXPERIMENTAL METHOD

Rats divided into groups of 8-10 animals were used. The rats were decapitated, the brain was quickly removed in the cold, and the tissues of the hypothalamus were pooled and homogenized in 0.32 M sucrose solution containing tranylcypromine (Parnate) in a concentration of 0.1 mg/ml. Unpurified mitochondrial fraction (UMF) was isolated after separation of the nuclei and large cell fragments by centrifugation at 12,000 g for 20 min. To isolate synaptosomes the suspension of UMF was layered on a sucrose density gradient of 0.8-1.2 M and centrifuged for 1 h at 100,000 g [9]. As a result, 3 separate fractions were obtained: the top layer containing myelin fragments and some light synaptosomes, the middle layer consisting almost entirely of synaptosomes, and the bottom layer consisting of pure mitochondria [6].

In each fraction the NA content and protein distribution were investigated. To determine NA a modified Euler's method [7] was used, including adsorption of NA on Al_2O_3 columns followed by oxidation by ferricyanide and spectrofluorometric determination of the oxidized products. The NA content was expressed in ng/mg protein, determined by Lowry's method [10].

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TABLE 1. Distribution of Protein (in mg/g tissues and in %) in Subcellular Fractions of Hypothalamus during Administration of Imipramine and Diazepam (mean results of 5-8 experiments; M±m)

Preparation	Dose (in mg/kg)	Myelin fragments		Synaptosomes		Pure mitochondria	
		mg/g	%	mg/g	%	mg/g	%
Control	—	2,38±0,16	10,4	2,75±0,15	10,3	21,1±1,1	79,4
Imipramine	10	2,52±0,27	10,0	3,10±0,10	10,9	19,8±1,1	79,1
Diazepam	5	2,28±0,19	10,2	2,90±0,23	13,7	17,2±1,2	75,3

TABLE 2. Effect of Imipramine and Diazepam on NA Content (in ng/mg protein and in %) in Subcellular Fractions of Hypothalamus (mean results of 5-8 experiments; M±m)

Preparation	Dose (in mg/kg)	Myelin fragments		Synaptosomes		Pure mitochondria	
		Fractions of ng/mg	%	Fractions of ng/mg	%	Fractions of ng/mg	%
Control	—	162,7±21,0	47,3	151,8±25,0	44,3	29,3±4,8	8,4
Imipramine	10	131,3±46,0	56,1	79,2±16,8	33,7	23,9±5,1	10,2
Diazepam	5	156,5±41,2	46,5	141,2±26,5	42,6	37,0±7,5	10,9

Imipramine (Tofranil) and diazepam (Seduxen) were injected intraperitoneally into the rats in doses of 10 and 5 mg/kg respectively. The animals were taken for the experiments 1 h after injection of the preparations.

EXPERIMENTAL RESULTS AND DISCUSSION

The distribution of protein in isolated fractions of the hypothalamus after separation in the sucrose gradient is given in Table 1.

The total protein content after fractionation was similar in the control and experimental series. More than 70% of protein was found in the pure mitochondria and the rest was distributed uniformly between the fractions of myelin fragments and synaptosomes. Similar values for the protein distribution, which according to data in the literature vary fairly widely depending on the technique of homogenization, have also been obtained by other workers [1].

The distribution of NA (Table 2) in the subcellular fractions was as follows. Both normally and after injection of the drugs the largest amount of NA was found in the fractions of synaptosomes and myelin fragments and only 8-10% was found in the fraction of pure mitochondria. Under the influence of imipramine, a marked decrease was observed in the NA content in UMF (234.4 ng/mg protein compared with 343.8 ng/mg protein in the control), mainly on account of a decrease in the concentration of mediator in the synaptosomes (79.2 ng/mg compared with 151.8 ng/mg in the control). This decrease was statistically significant.

In the experiments with diazepam, no appreciable effect of this substance was found on the content of adrenergic mediator either in the synaptosomes or in the other fractions of hypothalamic tissue.

Analysis of the literature shows that in some cases the views regarding the neurochemical nature of action of psychotropic drugs were formed on the basis of investigations of brain homogenates and undifferentiated structures. This applies to some extent to studies with imipramine and diazepam and it is evidently one of the reasons for the contradictory nature of the data on the mechanism of their action [4, 8]. Subcellular fractionation of hypothalamic tissue, as used in the present experiments, yielded a synaptosomal fraction with a relatively high NA concentration. In this way it was possible to investigate the NA content in a system free from other cell components (myelin, mitochondria) and to obtain direct proof of the effect of the drugs on the adrenergic mediator.

The results of these experiments showed that imipramine, while causing no morphological changes in the cell (as shown by the absence of change in protein distribution), definitely lowers the NA content in the synaptosomes. Changes in the other fractions were not statistically significant. It can accordingly be

concluded that imipramine has a direct effect on the adrenergic mediator, and this is in good agreement with the hypothesis of the role of the hypothalamus in the mechanism of action of this antidepressant [4]. The absence of any such effect by diazepam is evidence that adrenergic structures play no part in the mechanism of its action.

LITERATURE CITED

1. D. Biesold, in: Biochemistry and Function of the Nervous System [in Russian], Leningrad (1967), p. 115.
2. E. L. Dovedova and R. P. Porfir'eva, Farmakol. Toksikol., No. 1, 14 (1974).
3. V. V. Zakusov, The Pharmacology of Central Synapses [in Russian], Moscow (1973), p. 116.
4. I. P. Lapin and L. Kh. Allikmeta, in: Modern Psychotropic Drugs [in Russian], No. 2, Moscow (1967), p. 113.
5. R. P. Porfir'eva and S. S. Boiko, Byull. Éksperim. Biol. Med., No. 3, 70 (1973).
6. J. Eichberg et al., Biochem. J., 92, 91 (1964).
7. U. Euler and F. Lishaiko, Acta Physiol. Scand., 51, 348 (1961).
8. M. Fennessy and J. Lee, Arch. Internat. Pharmacol., 197, 37 (1972).
9. R. Levi and E. Maynert, Biochem. Pharmacol., 13, 615 (1964).
10. O. H. Lowry et al., J. Biol. Chem., 193, 265 (1951).