may be associated with the more rapid breakdown of adenine derivatives of the purines to inosinic acid, to inosine, and also possibly, to hypoxanthine.

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INHIBITION OF NORADRENALIN DEAMINATION BY VARIOUS ANTIDEPRESSANTS

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Changes in metabolism of serotonin or noradrenalin (NA) in the brain play an important role in the mechanism of action of antidepressants [8]. Such changes may be the result of disturbance of biosynthesis, storage, or (and) deamination of the amines. Incomplete correlation between inhibition of monoamine oxidase (MAO) and amine accumulation in the brain [6] may be due to the multiplicity of forms (heterogeneity) of MAO, the key enzyme of amine catabolism. According to the binary classification, MAO exists in the form of two types: A and B. Serotonin and NA are specific substrates of type A MAO, which is sensitive to low concentrations of chlorgyline [10]. Much greater inhibition of deamination of NA than of serotonin has been demonstrated under the influence of pyrazidol (pirlindol), an inhibitor of type A MAO, on rat brain mitochondria [4]. The possible existence of an independent binding site (or form of enzyme), responsible for NA deamination in the human brain [11], was indicated previously.

Imipramine, a reuptake inhibitor, inhibited deamination of serotonin and 2-phenylethylamine in the brain [2, 9], but the effect of this drug, and also of other antidepressants that are inhibitors of monoamine reuptake on NA deamination has not been investigated.

For the reasons described above, it was decided to study the effect of antidepressants with different structure and type of action on enzymic deamination of NA in the brain.

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TABLE 1. Effect of Antidepressants on Deamination of NA and Serotonin on Incubation with Bovine Brain Mitochondria (M ± m)

Preparation	Concentration, M	Inhibition of MAO activity, %	
		NA	serotonin
Control	—	0	0
Pyrazidol	10^{-5}	80±8	60±4
Inkazan	10^{-5}	60±7	70±7
Moclobamide	10^{-5}	45±5	50±5
Imipramine	10^{-5}	43±4	50±5
Compound 30	10^{-5}	27±3	55±6
Viloxazine	10^{-4}	44±4	1±0,1
Zimelidine	10^{-4}	35±3	5±0,5
Norzimelidine	10^{-4}	66±7	30±3

Legend. Results of four determinations are given. MAO activity of 2.5 ± 0.5 nmoles and 2.1 ± 0.2 nmoles serotonin/mg protein/min taken as 100%. Differences significant at the P = 0.05 level.

EXPERIMENTAL METHOD

Fragments of mitochondrial membranes from bovine brain, isolated by the standard method [5], were used as the source of enzyme. The velocity of the enzymic deamination reaction was judged from the quantity of ammonia set free [1]. The final concentration of NA bitartrate (from Serva, West Germany) in the sample was 13 mM, and of serotonin creatinine-sulfate (Serva) 4.4 mM. The inhibitors were dissolved in water or in 96% ethanol; in the latter case, diluting them further 100-fold with 0.01 M phosphate buffer, pH 7.4, and added to the samples, which were not preincubated. The alcohol concentration was taken into account in the control. Protein was determined by Lowry's method [7].

EXPERIMENTAL RESULTS

Data on the effect of antidepressants with different structure on oxidative deamination of NA and serotonin in bovine brain mitochondria are given in Table 1. Pyrazidol, which inhibited the reaction by 80% in a concentration of 10^{-5} M, was the most active inhibitor of NA deaminat-on. Close to it as regards degree of action on NA deamination in bovine brain was inkazan, whose inhibitory effect in the same concentration was 60%. Imipramine reduced the rate of NA deamination in bovine brain mitochondria by 50%, which was not inferior in this respect to the action of moclobamide, a benzamide derivative with antidepressant properties. Another benzamide derivative compound 30, depressed NA deamination in bovine brain much less strongly.

Data for compounds causing weaker inhibition of NA deamination are given for concentrations of 10^{-4} M. Viloxazine and zimelidine in this concentration (Table 1) inhibited noradrenalin deaminase activity of the mitochondria weakly (by 25-45%), and the inhibitory effect of norzimelidine was stronger, at 66%.

Some differences may be noted in the action of these antidepressants on oxidative deamination of two substrates of type A MAO, namely NA and serotonin. Pyrazidol, for instance, had a stronger antinoradrenalin-deaminase than antiserotonin-deaminase action (Table 1). The same result also has been shown for rat brain mitochondria [4]. Imipramine, inkazan, and moclobamide inhibited the deamination of these two type A MAO substrates in bovine brain equally. The effect of zimelidine, viloxazine, and norzimelidine on NA deamination was found in the same concentrations of the drugs as for serotonin deamination, but the experiments showed that the serotonin-deaminase activity of bovine brain mitochondrial MAO is less sensitive to the action of these antidepressants than noradrenalin-deaminase activity. The present writers found previously that many of the compounds tested are able to inhibit reverse synaptosomal transport of monoamines. Imipramine, for example, in a concentration of 50 µM, actively inhibited NA and serotonin uptake by brain synaptosomes, acting more selectively on serotonin transport (inhibition by 45 and 70%, respectively). Zimelidine and norzimelidine exhibited

even greater selectivity in relation to reverse transport of serotonin into nerve endings, whereas inkazan was found to be a selective inhibitor of this process, with no effect on NA uptake [3]. Viloxazine and pyrazidol inhibited reuptake of both mediators in rat brain by 30-40%, whereas moclobamide and compound 30, in the concentration tested (50 μM), did not change it [3].

It is thus difficult to discover correlation between inhibition of enzymic deamination of NA by the compounds studied and its uptake by brain synaptosomes, as in the case of deamination and reverse transport of serotonin. Correlation likewise was not found between the degree of action of the compounds on NA deamination and serotonin oxidation. This may evidently indicate the existence of different sites of the MAO active center for deamination of each of these substrates, despite the fact that both mediators are substrates of type A MAO.

It can be concluded from the results of this investigation that an important role in the neurochemical mechanism of the action of drugs such as pyrazidol, moclobamide, inkazan, and imipramine, is played not only by inhibition of the serotonin-deaminase reaction, but also, probably, by their effect on NA deamination in the brain. However, for some antidepressants (imipramine, pyrazidol) inhibition of reverse transport of NA into nerve endings is also, evidently, of great importance.

On the example of imipramine and pyrazidol, a combined action can thus be observed on enzymic and transport inactivation of NA in the brain. The noradrenergic component in the neurochemical spectrum of zimelidine, norzimeldine, and viloxazine is evidently not so strongly expressed as in the other drugs mentioned above, especially as regards deamination of this mediator; a more important role in the mechanism of the pharmacologic effect of these drugs may be played by their powerful inhibitory action on the process of synaptosomal reuptake of serotonin in the brain.

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