DIFFERENTIATION BETWEEN SECONDARY AND TERTIARY TRICYCLIC ANTIDEPRESSANTS IN FROGS

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Frogs (Rana temporaria) were used as the test object for detection of the serotonin-positive action of psychotropic drugs. The doses of the tertiary antidepressants (chlorimipramine, imipramine, amitriptyline) potentiating the sedative (inhibition of the righting reflex) and excitatory (twitching of the limbs) effects of phenelzine, alone or in conjunction with reserpine, were several times smaller than the corresponding doses of the secondary compounds (desmethylimipramine and nortriptyline). This agrees with results obtained by other workers regarding differences in the effects of tertiary and secondary compounds on indolealkylamine metabolism. None of the antidepressants tested potentiated the sedative action of amobarbital. The potentiating effect of the tricyclic antidepressants is linked with their serotonin-sensitizing effect.

Reserpine has a sedative action on frogs if they are first given monoamine oxidase inhibitors [7]. The tricyclic antidepressants render the sedative action of subthreshold doses of reserpine manifest [3]; characteristic twitching of the limbs is observed [3]. The phenomenon of synergism of the tricyclic antidepressants with reserpine in frogs, which correlates with activation of serotoninergic processes [5, 3], has been suggested as a test for screening the tricyclic antidepressants [4], whose thymoanaleptic action is evidently linked with their central serotonin-positive effect [8, 2]. It has been shown that the test on frogs can distinguish the tricyclic antidepressants from neuroleptic and cholinolytic drugs similar to them in their chemical structure and pharmacological activity.

In the investigation described below the object was to determine whether this test can be used to differentiate between different members within the group of the tricyclic antidepressants.

Tertiary (chlorimipramine, imipramine, amitriptyline) and secondary (dimethylimipramine [DMI], nortripytline) tricyclic antidepressants, differing in the intensity of their effects on serotonin metabolism, were studied for this purpose. For instance, the ability of the tertiary compounds to inhibit transmembrane serotonin transport into the presynaptic endings of neurons [9] and into platelets [10] decreases in their demethylated derivatives [9, 10].

EXPERIMENTAL METHOD

The investigation was carried out in the autumn-winter period on male frogs (Rana temporaria) living near Leningrad. During the experiments the animals were kept in groups of seven in plastic bowls 30 cm in diameter containing a little water. All the compounds were injected as aqueous solutions into the femoral and submandibular lymph sacs in a volume of 0.01 ml/g. Solutions of phenelzine and nortriptyline were made up from powders, and solutions of imipramine (melipramin), DMI (Pertofram), chlorimipramine (Anafranil), amitriptyline (triptizol), and reserpine (rausedil) from preparations packed in ampules.

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TABLE 1. Potentiation of Serotoninergic Effects in Frogs by Tricyclic Antidepressants

Compound	Minimal doses eliciting effect			
	sedative	excitatory	sedative	excitatory
	substances administered additionally			
	phenelzine (25 mg/kg) + reserpine (10 mg/kg)		phenelzine (25 mg/kg)	
Chlorimipramine	0,5	1,25	5,0	5
Imipramine	1,25	1,25	2,5	5
Amitriptyline	1,25	1,25	5,0	10
Desmethylimipramine	20,0	>20,0	20,0	>20
Nortriptyline	20,0	>20,0	20,0	>20

The tricyclic antidepressants were injected 1.5 after phenelzine (25 mg/kg) and 30 min before reserpine (10 mg/kg). Bearing in mind that a combination of phenelzine and reserpine in the above doses leads to same increase in the serotonin level in brain tissue as injection of phenelzine alone [5], in one series of experiments the effects of the tricyclic antidepressants were tested on frogs receiving phenelzine alone (25 mg/kg).

As a control of the specificity of the phenomena observed, the effects of large doses of antidepressants, either alone or in conjunction with amobarbital, which has a general inhibitory action on frogs [7], on the behavior of the animals were investigated.

The action of the compounds was assessed by the smallest effective doses of the tricyclic antidepressants which gave rise to the following statistically significant changes in the animals' behavior 4.5 h after their administration: a) inhibition of the righting reflex, a sedative effect [7, 3], was determined ten times in succession in each frog before and after injection of the compounds; b) the appearance of characteristic twitches of the limbs, an excitatory effect [5], was recorded in alternative form (present or absent). In each group the number of frogs in which these effects were observed was counted. Statistical analysis of the data was carried out by means of the X^2 criterion [1].

EXPERIMENTAL RESULTS

Tertiary antidepressants, in conjunction with phenelzine alone, were less effective than in conjunction with phenelzine and reserpine (Table 1). This may have been because reserpine, by liberating serotonin from the depots, increases the content of the functionally active amine. The identical degree of elevation of the serotonin level in the brain after administration of phenelzine with reserpine and of phenelzine alone may be attributed to the fact that mainly the content of amine in the depots was determined [5]. The results thus do not contradict the previous hypothesis that the synergism between tricyclic antidepressants and reserpine in experiments on frogs is associated with activation of serotoninergic processes [3]. Synergism of the compounds tested, with both reserpine and phenelzine, evidently reflects the serotoninpositive effect of the tricyclic antidepressants, as is confirmed by comparison of the effects of the tertiary and secondary compounds (Table 1).

The smallest effective doses of the tertiary antidepressants in both series of experiments (phenelzine + reserpine and phenelzine alone) were several times smaller than the corresponding doses of the secondary derivaties. The secondary and tertiary antidepressants themselves inhibited the righting reflex in the frogs. The minimal dose of chlorimipramine with a sedative action was 70 mg/kg, that of imipramine, DMI, and amitriptyline was 50 mg/kg, and of nortriptyline 40 mg/kg. Consequently, the differences between the effects of the tertiary and secondary antidepressants revealed by their administration in conjunction with phenelzine and reserpine does not correlate with the strength of their intrinsic inhibitory action. Other evidence of the specificity of the phenomenon of synergism of the tricyclic antidepressants with phenelzine and reserpine is given by the fact that in the present experiments the tricyclic antidepressants did not potentiate the sedative action of amobarbital (25 mg/kg).

The test on frogs can thus be used not only to differentiate the tricyclic antidepressants from neuroleptics and cholinolytics [4, 6], but also to distinguish those members of the group of tricyclic antidepressants which have the most marked serotoninpositive action.

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