Effect of dimethyl and monomethyl tricyclic antidepressants on central 5-hydroxytryptamine processes in the frog

We previously described a test for screening thymoleptic drugs. This test is based on measuring sedation (loss of the righting reflex) and of twitches of the extremities in the frog. Both effects are presumably related to activation of central 5-hydroxytryptamine (5-HT) processes (Lapin, Oxenkrug & others, 1970).

We now report our observations on the relation between chemical structure of tricyclic antidepressants and their activity in this test.

Male frogs (*Rana temporaria*) were used in our experimental procedure identical to that described earlier, except that the experiments were made in winter whilst the previous experiments were made in spring and summer. We used chlorimipramine, imipramine, amitriptyline and desipramine and nortriptyline. We measured the minimal doses that produced inhibition of the righting reflex and the appearance of twitches of the extremities 4 h after injecting reserpine.

Both sedation and twitches were observed when the elevation of brain 5-HT was 3-fold or more (Oxenkrug, Osipova & Uskova, 1970). Since an increase in brain 5-HT was produced by a combination of phenelzine (25 mg/kg) with reserpine (10 mg/kg) or more so by phenelzine alone (25 mg/kg) (Oxenkrug & others, 1970), one series of our experiments was done without reserpine (Table 1).

Table 1. *Potentiation of central 5-HT effects in the frog by dimethyl and monomethyl tricyclic antidepressants.*

			Minimal effective doses (mg/kg) for:					
	I*	T†	I	T	I	T	I	T
			after pretreatment with:					
			Phenelzine (25 mg/kg) +					
	Distilled water		reserpine (10 mg/kg)		Phenelzine (25 mg/kg)		Amylobarbitone (25 mg/kg)	
Drug								
Dimethyl compounds								
Chlorimipramine ..	70	0	0·5	1·25	5	5	70	0
Imipramine	50	0	1·25	1·25	2·5	5	50	0
Amitriptyline	50	0	1·25	1·25	5	10	50	0
Monomethyl compounds								
Desipramine	50	0	20	>20‡	20	>20‡	50	0
Nortriptyline	40	0	20	>20‡	20	>20‡	40	0

* Inhibition of righting reflexes.
† Appearance of twitches.
‡ If any.

Dimethyl antidepressants are much stronger than their monomethyl derivatives in enhancing central 5-HT processes in the frog (Table 1). Difference in the strength of potentiation (in the test of inhibition of the righting reflex) is not related to the sedative action of tricyclic antidepressants, as it can be seen from the ratio of sedation to potentiation. The same is also shown in the inability of antidepressants to potentiate, or to enhance, the sedative action of amylobarbitone. The minimal sedative dose of amylobarbitone in our experiments was 60 mg/kg.

Our results are also consistent with observations of others that the tertiary compounds have more pronounced influence on metabolism of indolealkylamines than have the secondary tricyclic antidepressants measured on uptake of 5-HT by the presynaptic membrane of serotoninergic neuron (Carlsson, 1970) and by blood platelets (Todrick & Tait, 1969).

Laboratory of Psychopharmacology,   G. F. OXENKRUG
Bekhterev Psychoneurological Research Institute,   I. P. LAPIN
Leningrad, U.S.S.R.
June 25, 1971

REFERENCES

CARLSSON, A. (1970).   *J. Pharm. Pharmac.*, **22**, 729–732.
LAPIN, I. P., OXENKRUG, G. F., OSIPOVA, S. V. & USKOVA, N. V. (1970).   *Ibid.*, **22**, 781–782.
OXENKRUG, G. F., OSIPOVA, S. V. & USKOVA, N. V. (1970).   In *The Serotoninergic Processes in Action of Psychotropic Drugs.*   Editor: Lapin, I. P., pp. 45–57.   Leningrad.
TODRICH, A. & TAIT, A. C. (1969).   *J. Pharm. Pharmac.*, **21**, 751–762.

The distribution of noradrenaline in the undivided spleen of the cat

Dearnaley & Geffen (1966) showed that it was more satisfactory to express the noradrenaline content of the cat spleen in terms of the deoxyribonucleic acid-phosphorus (DNA-P) content of that organ rather than in terms of tissue wet weight. Furthermore, when the relation between the noradrenaline contents, expressed per μmol DNA-P, of the anterior and posterior portions of the cat spleen was calculated, the posterior portion was found to contain an amount of noradrenaline that was equivalent to 90% of that in the anterior portion.

The distribution of the noradrenaline content of the anterior portion of cat spleens was found to be approximately $\log_{10}$ normal (Brown, Dearnaley & Geffen, 1967).

Experiments with the effects of several drugs on the noradrenaline content of the undivided spleen *in situ* (Abbs & Robertson, 1969, 1970; Robertson & Abbs, 1971) have been made and it was therefore reasonable to determine the normality of the distribution of noradrenaline in the whole spleen; the rankit method (Ipsen & Jerne, 1944; Bliss, 1967) was applied.

The experimental procedures have been previously described (Abbs & Robertson, 1970). Cat spleens were homogenized in an ice-cold sucrose medium and the noradrenaline and DNA-P contents were measured. Figures from twenty-six experiments were available for analysis.

Using the rankit method and an Elliot 4130 computer linked to a graph plotter, graphs were prepared and points plotted for the observations of the noradrenaline content of the spleen, expressed as ng per μmol DNA-P, without transformation and also with the following five transformations: x^2, $x^{\frac{1}{2}}$, x^0, $x^{-\frac{1}{2}}$, x^{-1}.

A straight line for each set of points was fitted by applying the method of least squares. The square root and logarithmic transformations gave reasonably linear trends but the best linear fit was found for those observations plotted without transformation (Fig. 1a). The mean and standard deviation for each form of the observations were interpolated from the six graphs using a procedure described by Ipsen & Jerne (1944) and were compared with calculated values (Table 1). Agreement between the interpolated and calculated means and standard deviations was closest with the untransformed figures but there was also good agreement for the square root and logarithmic functions.

As an attempt to differentiate between these possibilities, a second degree polynomial curve was fitted to each set of plotted points (Fig. 1b). In this series, the observations without transformation gave a graph which was almost rectilinear. The other graphs, however, were distinctly curvilinear indicating that the data in the transformations were not distributed normally.

Our results with the modified rankit method show that the criteria for normality