LETTERS TO THE EDITOR

The frog as a subject for screening thymoleptic drugs

Lapin, Osipova & others (1968) observed in frogs pretreated with the monoamine-oxidase inhibitor phenelzine that the tricyclic antidepressants imipramine and desipramine enhanced reserpine or 5-hydroxytryptophan effects–loss of the righting reflex (sedative action) and the appearance of the typical twitches of the extremities. The 5-hydroxytryptamine (5-HT) antagonist bromolysergide (BOL-148) prevented this potentiating effect. Oxenkrug, Osipova & Uskova (1970) reported that potentiation of reserpine effects by desipramine occurs only when the concentration of brain 5-HT was 3 or more times greater than that in the control. The concentration of adrenaline was unaffected. It was suggested that enhancement of reserpine effects in the frog reflects the central 5-HT potentiation of desipramine (or another agent with the similar action in this test).

This phenomenon was then used to examine the mechanism of action of the tricyclic antidepressants and agents with similar pharmacological action and chemical structure: neuroleptics, anticholinergic drugs and stimulants. All drugs, as aqueous solutions, were injected into thigh or mandibular lymph sacks. Frogs (*Rana temporaria*) were treated with phenelzine (25 mg/kg), 1·5 h later with desipramine (or test compound), and after another 30 min with reserpine (10 mg/kg). Four h after injection of reserpine the righting reflex was recorded, the number of successful attempts (from 10 trials) being counted. The Student *t*-test was used for statistical treatment of data. At the same time twitches of the extremities were tested as positive or negative, and the number of animals presenting twitches was recorded.

The antidepressants of the imipramine group: imipramine (5 and 10 mg/kg), desipramine (10 mg/kg), amitriptyline (10 mg/kg), chlorimipramine (10 mg/kg), prothiaden (10 mg/kg) and dibenzepin (10 mg/kg) enhanced the sedative action of reserpine. The twitches of extremities appeared in frogs treated with all these drugs except dibenzepin.

Chlorpromazine (10 mg/kg) and benactyzine (20 mg/kg) also enhanced the sedative effect of reserpine, but did not produce twitches. Chlorpromazine (5 mg/kg), promazine (10 mg/kg), haloperidol (2 mg/kg), trifluoperazine (2 mg/kg), atropine (20 mg/kg), amphetamine (5 and 10 mg/kg) and imidazol derivative AW-151129* (10 and 20 mg/kg) did not enhance the two effects of reserpine. AW-151129 appeared to be a compound with the pharmacological profile of tricyclic antidepressants, but it did not exert a thymoleptic effect in depressive patients (Stille, Lauener & others, 1968).

The data accord with the hypothesis that the thymoleptic effect is mediated through the activation of the central 5-HT processes (Carlsson, Corrodi & others, 1969; Lapin & Oxenkrug, 1969).

The phenomenon of potentiation of the reserpine effects in the frog may prove useful in the screening of thymoleptics.

* 5-(*p*-Chlorophenyl)-2,3,5,6-tetrahydro-imidazo (1,2-c) quinazoline.

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Molecular complexation of morphine and indol-3-yl sulphuric acid in the dog

During the course of investigation on morphine conjugates in the dog (Misra, Yeh & Woods, 1970) it was found that the methanolic eluate from Amberlite XAD-2 resin column on which urinary metabolites of morphine-*N*-methyl[^{14}C] had been adsorbed, on descending paper chromatography with the solvent systems n-butanol–acetic acid–water (I, 4:1:5 v/v organic phase; II, 35:3:10 v/v; III 100:4:24 v/v) consistently showed the presence of another iodoplatinate-positive radioactive spot having an Rf higher than that of free morphine and morphine conjugates. The Rf values of free morphine and this unknown radioactive spot in systems I-III were 0·53, 0·63; 0·43, 0·52; 0·38, 0·55 respectively. Paper and thin-layer chromatography using n-butanol-mineral acid or ammonia systems however showed the morphine spot only. Washing the methanolic residue of unknown product from Whatman 3 MM paper chromatograms with 4% K_2HPO_4 solution, saturated bicarbonate or ammonia solutions, and subsequent extraction with ethylene dichloride containing 30% n-amyl alcohol, gave an extract which showed the presence of morphine only. Similarly autoclaving the residue of unknown product with 2·4 N hydrochloric acid at 15 p.s.i. for 1 h, basification to pH 9, and solvent extraction showed a light indigo blue colour in the organic phase which on evaporation and paper chromatography showed the presence of morphine only.

A positive colour test for indol-3-yl sulphate with Ehrlich reagent (Decker, 1955; Rodnight, 1956) and morphine on acid or alkaline treatment suggested that the unknown radioactive product was a molecular complex of morphine and indol-3-yl sulphuric acid. Co-chromatography of the eluted unknown product with a synthetic morphine-indol-3-yl sulphuric acid complex prepared as described below substantiated this point. A single spot (Rf 0·52), positive to iodoplatinate and Ehrlich reagents, with a single peak of radioactivity coincidental to this spot was obtained using system III (Rf non-labelled morphine, 0·36). The complex isolated from Whatman 3 MM paper chromatograms with methanol and purified on neutral alumina column was a brownish hygroscopic powder of ill-defined melting point softening at 155–160° and melting at 175–178° (decomp.).

Molecular complexes of morphine, nalorphine, normorphine and tryptamine bases 1:1 with indol-3-yl potassium sulphate (indican) were prepared by the method of Boyland, Sims & Williams, 1956. Morphine complex: turns blue at 150°, m.p. 167–170° (decomp.), $C_{25}H_{26}N_2O_7S$, $\frac{1}{2}H_2O$, yield 55%. Nalorphine complex: m.p. 186–187° (decomp.), $C_{27}H_{28}N_2O_7S$, $\frac{1}{2}H_2O$, yield 80%. Normorphine complex: sinters 167°,