An antitussive test using guinea-pigs

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A review of methods used for screening potential new antitussive agents suggests that tests based on exposure of guinea-pigs to inhalation of irritants are commonly used and that sulphur dioxide, ammonia or acrolein have found most favour (Eddy, Friebel, Hahn & Halbach, 1969). Our choice during 10 years of testing has been an aerosol of citric acid, the irritant most often chosen for comparable tests in man.

Our apparatus is simple, inexpensive and designed to record the incidence of coughing in six guinea-pigs at a time. They are housed in a sectionalized Perspex box sealed with a readily detachable common front fitted with a rubber gasket. Each individually sealed compartment connects with (*a*) an aerosol source, (*b*) a small Marie tambour (Palmer, Model No. 3012) writing on a smoked drum and (*c*) a wash bottle containing alkali to absorb escaping aerosol. A single glass nebulizer (Agla S/376C) run at 5 lb/inch^2 may serve all six compartments through a six-way manifold without interference between recordings if the inlet to each compartment is suitably restricted (for example with 7 cm lengths of 2 mm diameter glass tubing).

In practice, coughs are counted over a 5 min period commencing 2·5 min after beginning to pass an aerosol of 20% citric acid. A total of thirty-six animals are used during the course of six runs, treatments and boxes being randomized. One or two groups serve as controls; their mean counts are usually 10–15. Provided animals of uniform weight and history are available, pre-calibration of their sensitivity is not recommended as was done for the ammonia exposure test of Winter & Flataker (1954). Some results using known drugs will be presented.

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Effects of bethanidine on responses to ergotamine and noradrenaline

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Sensitivity to intravenous ergotamine was determined in two situations of higher sensitivity to noradrenaline following treatment with bethanidine.

1. Responses of the nictitating membranes in each of four anaesthetized cats (pentobarbitone) were determined first to intravenous noradrenaline and then to ergotamine, using a logarithmically increasing series of doses. The interval between doses of 7–10 min allowed full recovery from the noradrenaline but not from the ergotamine responses. These cats were allowed to recover, given bethanidine (3 mg/kg subcutaneously) on each of 14 consecutive days and finally re-anaesthetized 18–24 h after the last dose for redetermination of nictitating membrane responses. Noradrenaline sensitivity increased about one hundredfold as in previous studies

(Green & Robson, 1965). Ergotamine sensitivity increased, but by only 2·5 times the mean equiactive doses before and after bethanidine treatment, being 20 and 50 μg respectively.

2. Dose/pressor response relationships were determined first for noradrenaline and then for ergotamine in pithed rats. Dosage was increased logarithmically and the interval between doses of 3–10 min was sufficient to allow recovery from the pressor effect of noradrenaline but not of ergotamine. For a pressor response of 40 mm Hg in eight control rats a mean of 0·42 (S.E. $\pm$0·09) μg noradrenaline or 9·1 (S.E. $\pm$1·8) μg ergotamine was required. In nine rats given bethanidine (10 mg/kg 85–95 min previously), when suppression of the pressor response to 4-(*m*-chlorophenylcarbamoyloxy)-2-butynyl-trimethyl ammonium chloride (McNeil A-343) showed that adrenergic nerves were blocked, comparable responses were produced by 0·056 (S.E. $\pm$0·008) μg noradrenaline or 2·39 (S.E. $\pm$0·35) μg ergotamine; doses approximately seven and four times less respectively than in controls. The dosages quoted for ergotamine represent about half the total amounts given. In five rats tested 18–24 h after 14 daily doses of bethanidine (10 mg/kg) when responses to McNeil A-343 were not impaired, noradrenaline and ergotamine sensitivity had not increased significantly. Shorter persistence of the effects of bethanidine in rats as compared with cats was found also by Follenfant & Robson (1970). The sensitivity to the pressor effect of noradrenaline of pithed rats was only half that of rats anaesthetized with urethane and pentobarbitone and given the ganglion blocking agent BW 139C55 (Green, 1956) either in the experiments reported by Robson (1967) or in five other animals tested by us. Administration of BW 139C55 was subsequently found approximately to double sensitivity in a proportion of pithed rats.

The sympathomimetic effects of low concentrations of ergotamine are attributable to direct activation of adrenoceptors (Rothlin & Cerletti, 1950; Innes, 1962). Ergotamine also has an affinity for the noradrenaline uptake mechanism, since in cat spleen it inhibits the uptake of exogenous and released noradrenaline (Dengler, Spiegel & Titus, 1961; Pacha & Salzmann, 1970). Potentiation by bethanidine of ergotamine as of noradrenaline may therefore be largely attributable to inhibition of uptake mechanism allowing greater concentrations to reach adrenoceptors. That sensitivity to ergotamine was less elevated than to noradrenaline suggests that uptake mechanisms usually remove a smaller proportion of ergotamine than of noradrenaline.

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