The effect of ethanol on the metabolism of amphetamine by the rat

In the course of an investigation into the effect of ethanol on the metabolism of amines we have studied the effect of pretreatment with ethanol on the metabolism of (±)-[2-^{14}C]amphetamine. Male Sprague-Dawley rats, 150 g, housed in metabolism cages received ethanol, 5 g/kg, as a 25% solution (v/v) by stomach tube followed 30 min later by (±)-[2-^{14}C]amphetamine sulphate (1·6 mCi/mmol), 5 mg/kg, intraperitoneally in 0·5 ml of isotonic saline. Control animals received an appropriate volume of isotonic saline by stomach tube and the same dose of (±)-[2-^{14}C]amphetamine sulphate. The pH of the urine was measured immediately after voiding, and the urine frozen. The urinary pH (5·5–6·8) was not altered by ethanol treatment. Urine was chromatographed in two dimensions on Whatman 3MM paper in n-butanol–acetic acid–water (4:1:1 v/v) followed by isopropanol–ammonia–water (8:1:1 v/v). The metabolites, located by autoradiography, were cut out and the radioactivity counted in a Packard Model 3375 liquid scintillation counter. Amphetamine, *p*-hydroxyamphetamine and *p*-hydroxyamphetamine glucuronide accounted for over 85% of the urinary radioactivity. Amphetamine and total *p*-hydroxyamphetamine were also determined by the method of Axelrod (1954) after treatment of the urine with β-glucuronidase. Amphetamine was further determined by isotope dilution and recrystallization to constant specific activity. The identification of *p*-hydroxyamphetamine glucuronide was confirmed by hydrolysis of the eluate from chromatograms with β-glucuronidase which converted it quantitatively to *p*-hydroxyamphetamine. Recovery of radioactivity in the urine in the first 24 h after dosing with amphetamine had a mean value of 77% for both control and ethanol-treated animals. The percentage of the urinary radioactivity present as amphetamine was greatly increased and that as free and conjugated *p*-hydroxyamphetamine greatly reduced by ethanol pretreatment: control and ethanol pretreated values being, respectively, 16·5 ± 5·28 and 65·4 ± 6·91 for amphetamine ($P < 0{\cdot}001$); 7·3 ± 3·60 and 2·4 ± 1·49 for *p*-hydroxyamphetamine ($P < 0{\cdot}005$); 61·8 ± 4·04 and 20·9 ± 5·99 for *p*-hydroxyamphetamine glucuronide ($P < 0{\cdot}001$) (means for 6 animals ± s.d.). The proportion of *p*-hydroxyamphetamine excreted in the free form (about 10% of the total *p*-hydroxyamphetamine) was the same in each group.

Ethanol pretreatment has a profound effect on the pattern of metabolism of noradrenaline, 5-hydroxytryptamine and tyramine (Davis, Brown & others, 1967, 1967a; Tacker, Creaven & McIsaac, 1969). However, these amines are deaminated by monoamine oxidase and the effect of ethanol is to increase the reduction of the intermediate aldehyde so formed. Amphetamine is metabolized in the rat largely by hydroxylation of the benzene ring (Axelrod 1954) so that no effect of ethanol on its metabolism would be expected. Whether the observed effect of ethanol on amphetamine metabolism is specific or is a general effect on aromatic hydroxylation, and whether the metabolic interaction of these two commonly abused drugs occurs also in man, is still unknown since the pattern of amphetamine metabolism in man is different from that in the rat (Dring, Smith & Williams, 1966).

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Observations on some actions of ergometrine, noradrenaline and dopamine on the guinea-pig vas deferens and on the rabbit jejunum

Some of the effects of dopamine may be brought about by an action on specific dopamine receptors as opposed to an action on α- or β-adrenergic receptors (McDonald & Goldberg, 1964; Eble, 1964; Rossum, 1965, 1966; Goldberg, Sonneville & McNay, 1968; Woodruff & Walker, 1969). In the brain of the snail, *Helix aspersa*, dopamine hyperpolarizes and inhibits some of the neurons by an action on specific dopamine receptors (Woodruff & Walker, 1969) and this action of dopamine is blocked by low concentrations of ergometrine, less effectively by α-blocking agents (Walker, Woodruff & others, 1968). Ergometrine has little or no α-blocking activity (Brown & Dale, 1935).

Cumulative concentration–effect curves were obtained for dopamine and for (—)-noradrenaline on the guinea-pig vas deferens, using the method of Rossum (1963). The mean pD_2 values were 4·6 for dopamine, and 5·6 for noradrenaline. Ergometrine maleate 10^{-6} to 5×10^{-6} M potentiated the response to noradrenaline (Fig. 1A), taking the form of an increase in the maximum effect obtainable, which rose to between 120% and 200% of control values. In Fig. 1A, there is also seen a shift to the left of the concentration–effect curve, but in other experiments in which ergometrine produced either no shift of the concentration–effect curve or a slight shift to the right, there was still seen an increase in the maximum response to noradrenaline. This action of ergometrine was reversed on washing. In contrast to its action on the noradrenaline response, ergometrine 2×10^{-6} to 10^{-5} M caused a decrease in the maximum effect of dopamine on the vas deferens. Over the lower concentration ranges of dopamine ergometrine caused potentiation of the response. Ergometrine alone had no effect on the vas deferens in concentrations up to 10^{-4} M.

Concentration–effect curves were obtained also on the isolated rabbit jejunum, using the method described by Rossum (1965). The mean pD_2 values obtained were 4·8 for dopamine and 7·0 for noradrenaline. In the presence of an amount of ergometrine (2×10^{-6} M), which itself had no effect on rhythmic activity, noradrenaline in concentrations of from 3×10^{-8} M to 10^{-6} M caused an increase in the amplitude of the spontaneous contractions instead of the usual decrease (Fig. 1B). Higher concentrations of noradrenaline in the presence of ergometrine caused the usual inhibition, with a small shift to the right of the concentration–effect curve, but with no change in the maximum effect obtainable (Fig. 1B). Similar results were obtained with dopamine as the agonist, with which ergometrine was less effective in reversing the inhibitory action of low concentrations, but caused a greater shift to the right of the concentration–effect curve. Ergometrine in concentrations greater than 10^{-5} M had a variable, but generally inhibitory, action on rhythmic activity.

One possible explanation of our observation on the rabbit jejunum is that ergometrine uncovers an excitatory action of noradrenaline, mediated through different receptors, perhaps also sympathomimetic. The mechanism of action of ergometrine on the vas deferens could possibly be similar to that suggested by Barnett, Greenhouse & Taber (1968) for other compounds on the rat vas deferens.