Effects of drugs on human foetal intestine: a preliminary investigation

In an attempt to study the pharmacological aspects of the development of smooth muscle we have investigated the effects of drugs on the isolated small intestine of the human foetus. After hysterotomy, the gastrointestinal tract was removed from foetuses of gestational age between 11 and 25 weeks, and stored in Krebs solution at 4°. Within 4–17 h after removal, a short length (2–3 cm) of small intestine was mounted in a 10 ml organ bath containing Krebs solution at 37° and the tone and movements of the longitudinal muscle recorded with an isometric transducer under a tension of 1–2 g. The tissue was allowed to equilibrate for 2 h before the application of drugs. Drug concentrations are expressed as the final bath concentration of base in g/ml.

Segments of small intestine from over 70 foetuses have been examined and most of these showed spontaneous activity throughout the experiment. All tissues contracted in the presence of acetylcholine in concentrations between 10^{-8} and 10^{-6}; the sensitivity did not appear to be related to gestational age but the more mature tissue was capable of developing a greater tension. The response to acetylcholine was potentiated by eserine (4×10^{-8}) and inhibited by atropine (4×10^{-8}), but not by hexamethonium (3×10^{-6}) suggesting that acetylcholine was acting on muscarinic receptors.

Histamine (2×10^{-8}–2×10^{-6}) caused contractions only in 9 out of 12 tissues and a biphasic response, relaxation followed by contraction, in the others. The histamine response appeared to be dependent on the maturity of the foetus because the biphasic response was observed only in tissues from foetuses of gestational age of 20 weeks or more. In the oldest tissue studied, from a 25-week foetus, histamine (4×10^{-8}) produced a relaxation, but later in the experiment a biphasic response was obtained. Neither the relaxation nor contraction was blocked by hexamethonium (5×10^{-6}) but both were blocked by mepyramine (10^{-6}).

The response to 5-hydroxytryptamine (5-HT) (2×10^{-8}–4×10^{-7}) also appeared to be dependent upon the age of the tissue. The longitudinal muscle from foetuses aged between 14 and 18 weeks contracted to this drug whilst a biphasic response of relaxation and contraction was observed in tissue from six 20-week foetuses. Responses to 5-HT were unaffected by hexamethonium (5×10^{-6}).

Noradrenaline (10^{-8}) (Fig. 1), phenylephrine (10^{-5}), adrenaline (10^{-7}) and isoprenaline (10^{-6}) inhibited spontaneous activity and caused relaxation in all the smooth muscle preparations of foetal intestine examined. The response to noradrenaline was blocked completely by propranolol (10^{-6}), reduced by phentolamine (10^{-5}) and unaffected by hexamethonium (5×10^{-6}) or guanethidine (10^{-5}) (Fig. 1).

The actions of nicotine, and the nicotine-like ganglionic stimulant dimethylphenylpiperazinium, were studied on most preparations; their effects were qualitatively similar. Low concentrations (6×10^{-7}) of nicotine usually produced a relaxation which was blocked by hexamethonium (5×10^{-6}), guanethidine (10^{-5}) (Fig. 1) or propranolol (10^{-6}) but in about 10% of the tissues a contraction was elicited which was blocked by atropine (4×10^{-8}). With higher doses of nicotine (5×10^{-5}), the longitudinal muscle relaxed and then contracted whilst, with doses greater than 10^{-4}, only a contraction was observed; the nicotine-induced contraction was unaffected by hexamethonium (5×10^{-6}), atropine (4×10^{-7}) and mepyramine (10^{-6}). The relaxation obtained with a low dose of nicotine (10^{-6}) could be reduced by the prior application of a higher dose (5×10^{-5}) of the drug. The duration of a response to nicotine appeared to be dependent on gestational age. In muscle from young foetuses

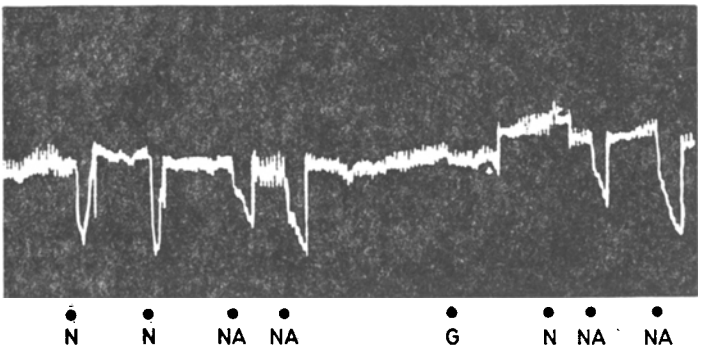

FIG. 1. Small intestine from 20-week foetus. Responses to nicotine (N) 6×10^{-6}, $1{\cdot}2 \times 10^{-5}$ and noradrenaline (NA) 5×10^{-8}, 10^{-7} before the administration of guanethidine (G) 10^{-5} for 8 min. After the guanethidine, the response to nicotine (N) 6×10^{-6} has been blocked whilst the responses to noradrenaline (NA) are unaltered. The relaxation to the first dose of nicotine is equivalent to a change in tension of 0·5 g.

(11–14 weeks) the offset of the effect was slow and unless an interval of 20 min elapsed between successive doses of the same amount of nicotine the response decreased. By contrast, in tissue from more mature foetuses (e.g. 20 weeks) consistent responses could be obtained by adding the drug at 6 min intervals.

In this study we have shown that longitudinal muscle from human foetal small intestine is capable of responding to acetylcholine, amines and ganglion-stimulants and that there may be a change in the response to histamine and 5-hydroxytryptamine at about 20 weeks. This extends the work of Boreus (1967, 1968) who reported that the muscarinic receptor is fully developed in the ileum of the 12-week human foetus.

It is of interest to compare the present results with those of other authors obtained with adult human small intestine. Thus, Bennett (1965) reported that histamine most frequently produced a relaxation but that contractions and biphasic responses were also seen; in the foetal tissue the tendency was for muscle from 14–18 week foetuses to contract whilst that from more mature foetuses gave a biphasic response. In adult tissue, 5-HT produced a contraction (Fishlock, 1964; Bennett, 1965; Whitney, 1965) as in tissue from younger foetuses but, with muscle from older foetuses, a biphasic response was seen. The relaxation to low doses of nicotine by the foetal muscle corresponds with the response of adult tissue (Bennett, 1965; Fishlock & Parks, 1966). This relaxation could be prevented by the presence of a ganglion blocker, a post-ganglionic sympathetic nerve blocker or a β-adrenoceptor blocker which suggests that the response involves release of an adrenergic mediator. This contrasts with the suggestion of Burn (1968) that the sympathetic innervation of the rabbit intestine changes from cholinergic to adrenergic between the third and eighth day after birth.

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Relation between drug elimination kinetics in intact animals and isolated perfused liver systems: phenylbutazone

Recent studies have shown the usefulness of the isolated perfused liver for investigations of the effects of enzyme induction and of distribution of drug on the drug's metabolism (von Bahr, Alexanderson & others, 1970; Nagashima, Levy & Sarcione, 1968; Levy & Nagashima, 1969). If a drug is eliminated only by biotransformation in the liver, good agreement between its *in vitro* and *in vivo* elimination rate constants may be obtained by correcting for the difference in drug distribution (liver: extrahepatic sites) in the perfused liver system and in the intact animal (Nagashima & others, 1968). This approach has been used successfully with bishydroxycoumarin and has yielded similar "true" elimination rate constants *in vitro* and *in vivo* even though the "apparent" elimination rate constants in perfused rat liver systems were three to four times higher than in the intact animals (Nagashima & others, 1968). The results to be reported here show that there is also a good quantitative correlation between *in vitro* and *in vivo* elimination rate constants in animals pretreated for various lengths of time with phenobarbitone, a potent microsomal enzyme inducing agent.

The elimination rate constant of phenylbutazone was determined in male Sprague-Dawley rats weighing 200 to 300 g and in isolated perfused livers obtained from similar animals. The intravenous dose (50 mg/kg) and the amount of drug in the

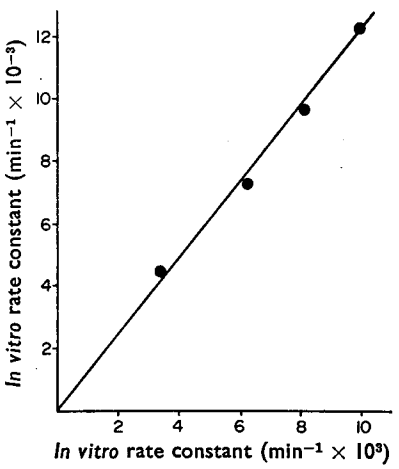

FIG. 1. Relation between *in vitro* and *in vivo* elimination rate constants of phenylbutazone in isolated perfused liver and in intact rats, respectively. From left to right, the data points represent values from control animals and from animals pretreated for 1, 2, and 3 days with phenobarbitone. The results are calculated from the experiments shown in Table 3 in the work of von Bahr & others. The points represent the mean values in these experiments.