Maximal responses in guinea-pig isolated ileum preparations: influence of longitudinal and circular muscle

Segments of the guinea-pig ileum prepared as described by Magnus (1904) are frequently used for the analysis of drug-receptor mechanisms involving agonists and antagonists. The maximal contraction (E_{max}) attainable with an agonist is an essential parameter for characterizing the effects of a drug in terms of affinity, intrinsic activity (Ariëns, Simonis & van Rossum, 1964) and efficacy (Stephenson, 1956). Preliminary investigations of the activities of choline and related compounds were made by using terminal segments of the guinea-pig ileum bathed in Tyrode solution at 37°. Cumulative concentration-effect curves (van Rossum & Ariëns, 1959) were obtained using an isotonic frontal writing lever (magnification × 10, and weighting 1 g) on a smoked drum.

Studies with choline and tetramethylammonium (TMA) which possess both nicotinic and muscarinic receptor stimulant properties (Triggle, 1965), showed that the maximal responses obtained with these compounds could be increased in the presence of the ganglion blocking drug hexamethonium. This unexpected effect was further evaluated using other drugs and procedures known to affect acetylcholine release. The mean maximal responses (± s.e.) of choline and TMA expressed as intrinsic activity (α), where α for acetylcholine is taken as unity, were 0·84 ± 0·05 for choline and 0·97 ± 0·10 for TMA. The mean concentrations (± s.e.) required to produce 50% of these maximal responses (EC50) were 0·09 ± 0·01, 56·0 ± 8·9 and 4·02 ± 1·55 μg/ml for acetylcholine, choline and TMA respectively. Table 1 shows the effects of various drugs and procedures on maximal responses and on EC50 concentrations for acetylcholine, choline and TMA. These values are expressed as percentage changes from control values obtained in Magnus preparations at 37°. Changes in EC50 values were calculated with respect to that response obtained under control conditions in order to avoid the influence of altered maximal responses on this parameter.

Concentrations of hexamethonium (12·5 μg/ml) which were sufficient to abolish responses to nicotine (2 μg/ml) produced only a small shift to the right of the concentration-effect curves to choline and TMA as shown by an increase in EC50 values. It would therefore appear that the predominant stimulant activity of choline, like TMA (Trendelenburg, 1961), does not involve nicotinic receptor stimulation in this tissue. In addition this treatment with hexamethonium produced increases of approximately 40% in the maximal responses attained with choline and TMA. Similar increases in maximal response were produced in the presence of morphine (0·5 μg/ml) and in tissues cooled to 25° for 15 min. These treatments are known to inhibit responses mediated indirectly through acetylcholine release in the guinea-pig ileum (Paton, 1957; Innes, Kosterlitz & Robinson, 1957). Atropine (12·5 ng/ml) shifted curves to three agonists to the right but did not increase their maximal responses.

Nicotinic receptor stimulation has been shown to produce a hexamethonium-sensitive release of catecholamines in various tissues (see Burn & Rand, 1965; Rand & Stafford, 1967, for references). However, catecholamine release was not responsible for the "inhibited" maximal responses to choline and TMA in the Magnus preparation at 37° as E_{max} was unaltered in the presence of adrenoreceptor blockade produced by a combination of phentolamine (0·1 μg/ml) and propranolol (0·1 μg/ml).

Careful observation of the ileal segments showed that at the higher concentrations used in the concentration-effect curves, choline and TMA produced a spasm within the tissue which was seen on the trace as irregular oscillations. TMA was more potent than choline in this respect. The spasm appeared to involve the circular

Table 1. *Effects of various treatments on % changes in maximal responses and EC50 values to acetylcholine, choline and tetramethylammonium.* Mean values (± s.e.) from 4 to 6 experiments of % change in maximal responses (E_{max}) and concentrations required to produce 50% of the control maximal response (EC50, cont) for acetylcholine, choline and tetramethylammonium (TMA) in Magnus preparations of the isolated guinea-pig ileum at 37°. Control values (Cont) and values obtained in the presence of hexamethonium (Hex, 12·5 μg/ml), morphine (Mor, 0·5 μg/ml) and adrenoreceptor blockade (α/βA) with phentolamine (0·1 μg/ml) and propranolol (0·1 μg/ml), and in tissues cooled to 25° (25°) are indicated. Values obtained in longitudinally cut preparations at 37° (acetylcholine = 100%) are also shown (L. cut, 37°).

		Magnus preparation (% change)					
		Cont	Hex	Mor	25°	α/βA	L. cut 37°
Acetylcholine	E_{max}	100	97 ± 5	88 ± 5	70 ± 9	95 ± 5	100
	EC50 (cont)	100	135 ± 14	190 ± 28	185 ± 37	145 ± 18	136 ± 14
Choline	E_{max}	100	147 ± 17	133 ± 5	124 ± 16	120 ± 2	168 ± 13
	EC50 (cont)	100	251 ± 48	315 ± 84	491 ± 62	109 ± 4	130 ± 28
TMA	E_{max}	100	137 ± 14	136 ± 19	124 ± 14	102 ± 2	152 ± 20
	EC50 (cont)	100	330 ± 10	450 ± 45	438 ± 38	135 ± 8	214 ± 36

muscle as evidenced by tight constrictions within the segment used. The possibility therefore existed that the "inhibited" maximal response might be due to a mechanical factor involving circular muscular contraction. Under experimental conditions where the maximal response was increased, no such constrictions within the ileal segments were noted.

To assess the influence of circular muscle activity on longitudinal muscle contractions, concentration-effect curves were produced in intact preparations, after which the ileal segments were cut longitudinally and curves to the agonists were re-established. In these cut preparations the maximal responses and EC50 values for acetylcholine were similar to those obtained in the intact preparation. In the cut preparations the maximal responses attained with choline and TMA with respect to acetylcholine (= 100%) were similar to, or greater than, those obtained in intact preparations at 37° which had been pretreated with morphine or hexamethonium or which had been cooled to 25°. Hexamethonium or morphine pretreatment had little further effect in enhancing maximal responses to choline and TMA in the cut preparations.

These results suggest that choline and TMA are producing differential concentration-dependent effects in the longitudinal and circular muscle coats of the guinea-pig ileum. The longitudinal muscle appears to be the most sensitive to muscarinic receptor stimulation. Higher concentrations are required for stimulation of circular muscle by choline and TMA, either directly, or indirectly through acetylcholine release. Brownlee & Harry (1963) have previously shown that there are differences in the sensitivities of strips of longitudinal and of circular muscle of the guinea-pig ileum to a wide variety of agonists.

The results of the present experiments show a limitation in the use of Magnus preparations of the guinea-pig ileum. Estimates of intrinsic activity or values of E_{max} which are essential for calculating other parameters, may be undervalued

because of mechanical inhibition of isotonic longitudinal muscle shortening through circular muscle activity.

Department of Pharmacology,
University of Melbourne,
Parkville, Victoria 3052,
Australia.

A. R. POMEROY
C. RAPER

June 9, 1971

REFERENCES

ARIËNS, E. J., SIMONIS, M. & VAN ROSSUM, J. M. (1964). *J. mednl Chem.*, **3**, 119.
BROWNLEE, G. & HARRY, J. (1963). *Br. J. Pharmac. Chemother.*, **21**, 544–554.
BURN, J. H. & RAND, M. J. (1965). *Ann. Rev. Pharmac.*, **5**, 163–182.
INNES, I. R., KOSTERLITZ, H. W. & ROBINSON, J. A. (1957). *J. Physiol., Lond.*, **137**, 396–409.
MAGNUS, R. (1904). *Pflügers Arch. ges. Physiol.*, **102**, 123–151.
PATON, W. D. M. (1957). *Br. J. Pharmac. Chemother.*, **12**, 119–127.
RAND, M. J. & STAFFORD, A. (1967). *Physiol. Pharmac.*, **3**, 1–95.
STEPHENSON, R. P. (1956). *Br. J. Pharmac. Chemother.*, **11**, 379–393.
TRENDELENBURG, U. (1961). *Arch. exp. Path. Pharmak.*, **241**, 452–466.
TRIGGLE, D. J. (1965). *Chemical Aspects of the Autonomic Nervous System*, p. 83, London: Academic Press.
VAN ROSSUM, J. M. & ARIËNS, E. M. (1969). *Archs int. Pharmacodyn. Thér.*, **118**, 418–446.

Analysis of the supersensitivity to noradrenaline induced by amphetamine in the isolated vas deferens of the rat

The development of supersensitivity in sympathetically innervated tissues may be of two different types. One type is specific to noradrenaline or other closely related sympathomimetic amines and dependent on a presynaptic mechanism, probably an impairment of the first step of the uptake process (Trendelenburg, 1963, 1966).

The second type of supersensitivity is non-specific and seems to be linked to a postsynaptic mechanism: modifications in the physiological state of the responding cells (Hudgins & Fleming, 1966; Westfall & Fleming, 1968a, b), or a change in the configuration of the adrenergic receptors (Carrier & Holland, 1965; Varma, 1966; Barnett, Greenhouse & Taber, 1968; Reiffenstein, 1968). Amphetamine is known to inhibit the uptake of noradrenaline (Axelrod, Hertting & Potter, 1962; Burgen & Iversen, 1965; Iversen, 1965, 1967; Häggendal & Hamberger, 1967). Recently it was suggested that amphetamine induces presynaptic supersensitivity to noradrenaline (de Moraes, Carvalho & Wherle, 1970). This report describes an investigation of the specificity of the amphetamine-induced change in sensitivity to noradrenaline.

Vasa deferentia of rats, 200–250 g, were mounted in Krebs-Ringer bicarbonate solution (de Moraes, Carvalho & Wherle, 1970). Dose-response curves to noradrenaline were made on each preparation before and after treatment with amphetamine for 20 min. Horizontal shifts of the log dose-response curves were measured at the level of the EC50. The same general procedure was employed to obtain the dose-response curves to methoxamine. Since it could not be obtained on the same vas deferens, because high concentrations of methoxamine induced spontaneous motility, vasa deferentia from the same rat were used to obtain EC50s. The error introduced is very small as vasa deferentia from the same animal had similar EC50s for methoxamine.

To measure adrenergic blocking activity, the pD'_2 value for noradrenaline-