phosphate buffer pH 7·35 is $2·74 \times 10^{-1} \pm 0·04 \times 10^{-1}$ h^{-1} ($r = 0·9911$, $n = 11$). Thus, the lower stability of phenylacetic mustard in albumin solution compared with chlorambucil is most likely due to the fact that phenylacetic mustard is bound to a less extent to albumin.

The binding of chlorambucil ($8·2 \times 10^{-5}$ M) in plasma from five healthy drug-free volunteers was 98·98 ± 0·05%, the mean albumin concentration being 46 mg ml^{-1}. Albumin seems to play a major role for the binding of chlorambucil in plasma since this is in good agreement with the values obtained in buffer solutions containing albumin (99·06 ± 0·15; albumin concentration 40 mg ml^{-1}, pH 7·35). The degradation half life was 17·78 ± 1·58 h at 37 °C which is in agreement with the value obtained in an albumin solution (15·8 ± 0·2 h, 45 mg ml^{-1} of albumin, pH 7·35, 37 °C).

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Nicotinic release of noradrenaline in the presence of tetrodotoxin from sympathetic nerve terminals in rabbit isolated atria

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It has been suggested that nicotinic cholinoreceptor agonists release noradrenaline in sympathetically innervated tissues by initiating action potentials in the terminal axons. The main evidence for this is that during exposure of sympathetically-innervated tissues to nicotinic agonists, antidromic impulses have been detected in sympathetic nerve trunks (Ferry 1963; Cabrera et al 1966; Davey et al 1968; Haeusler et al 1968; Krauss et al 1970; Bevan & Haeusler 1975). The role of propagated action potentials in these nicotinic responses has been examined in experiments with tetrodotoxin, which selectively blocks axonal conduction in various nerve-muscle preparations (Gershon 1967); however, these experiments have yielded conflicting results. Thus it has been found by some workers that tetrodotoxin blocks the responses of isolated tissues to nicotinic agonists (Bell 1968; Endoh et al 1970), whereas others did not observe a blocking action (Haeusler et al 1968; Krauss et al 1970; Su & Bevan 1970; Westfall & Brasted 1972; Fozard & Mwaluko 1976); furthermore, Furchgott et al (1975) found that tetrodotoxin blocked the responses to low but not to high concentrations of nicotine in the rabbit ear artery.

In the present experiments, the effects of tetrodotoxin were examined on noradrenaline release from rabbit isolated atria elicited by a range of concentrations of nicotine and by electrical field stimulation of the sympathetic nerve terminals. The efflux of tritium label from the tissue after labelling the noradrenaline stores with (−)-[^{3}H]noradrenaline was taken as the index of noradrenaline release. The atria were exposed to nicotine (10, 50 or 100 μM) for 3 min or field stimulation (1 ms monophasic square waves of supramaximal voltage at 5 Hz for 30 s) in either the absence or presence of tetrodotoxin (0·9 μM). The experimental details are as described by Sarantos-Laska et al (1980). Nicotine- or stimulation-induced efflux of tritium label was calculated by subtracting the efflux of radioactivity determined immediately before stimulation or exposure to nicotine from that determined during these procedures. The nicotine- and stimulation-induced effluxes of radioactivity were expressed as percentages of the tissue content of radioactivity immediately before each period of evoked release.

The results are summarized in Table 1. The amounts of noradrenaline released from the atria by field stimulation and by the low concentration of nicotine (10 μM) were similar, and in both cases the effects were substantially reduced by tetrodotoxin, to 10 and 39%, respectively, of control values. The higher concentrations of nicotine (50 and 100 μM) released much greater amounts of noradrenaline, and in these cases tetrodotoxin failed completely to reduce the effects.

The present findings that tetrodotoxin blocked the noradrenaline-releasing action of a low but not of high concentrations of nicotine are in accord with those of Furchgott et al (1975) who measured contractile responses of the rabbit ear artery, but not in accord with the results of Fozard & Mwaluko (1976) who did

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Table 1. Percentage release of tritium label from rabbit atria previously incubated in [^{3}H]noradrenaline induced by nicotine in three concentrations and by electrical field stimulation in the absence and presence of tetrodotoxin. The standard error of the means (s.e.m.) and the number of experiments (n) are shown in each case.

	Nicotine			Electrical field stimulation 5 Hz, 1 ms Supramaximal voltage for 30 s
	10 μM	50 μM	100 μM	
Control	0·96 ± 0·15 (5)	6·31 ± 0·14 (3)	15·98 ± 2·70 (5)	1·06 ± 0·13 (5)
Tetrodotoxin (0·9 μM)	0·37 ± 0·11* (5)	8·04 ± 2·5 (5)	23·3 ± 3·08 (4)	0·11 ± 0·04* (5)

* $P < 0·05$, Student's t-test.

not see an inhibitory action of tetrodotoxin with a large range of nicotinic agonist concentrations in rabbit heart. The marked differences in the amounts of noradrenaline released by low and high concentrations of nicotine, and the difference in susceptibility to tetrodotoxin, suggests that the mechanism of action of nicotine depends on its concentration. The reason for this may be as outlined by Furchgott et al (1975) (see also review by Starke 1977) for the noradrenaline-releasing action of nicotinic agonists: with low concentrations, tetrodotoxin-sensitive action potentials are initiated in the axon terminals; with high concentrations, release of noradrenaline is produced by tetrodotoxin-insensitive local depolarization of the axon terminals which is presumably associated with a generalized increase in cation permeability allowing an influx of extracellular calcium ion.

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