Excitatory effect of tetrodotoxin on an isolated smooth muscle organ

Tetrodotoxin has been shown to abolish action potentials by interfering with sodium conductance in nerve fibres (Narashi, Moore & Scott, 1964). Smooth muscle reactivity is not affected by this action and thus tetrodotoxin is recommended as a convenient "denervating" agent (cf. Gershon, 1967) in experiments on isolated innervated smooth muscle organs. The present report describes a stimulant action of tetrodotoxin in the isolated cat sphincter of Oddi.

It is possible to isolate the cat sphincter of Oddi from surrounding duodenal tissue and use it for experiments *in vitro*. The sphincter is used as a tubal preparation mounted longitudinally in oxygenated Krebs solution of 37° (Persson, 1971). The sphincter exhibits spontaneous rhythmic activity which is shown as simultaneous longitudinal activity and increased resistance to flow through the isolated sphincter (Fig. 1) when it is constantly perfused at a rate of 3 ml/h. The activity of the sphincter is not affected by atropine or by phenoxybenzamine and is present in the sphincter taken from reserpinized cats (Persson, 1971).

Tetrodotoxin (812093 B Sankyo Japan) (0·1–1 μg/ml) did not prevent the activity of the isolated sphincter of Oddi (Persson, 1971) but caused the sphincter to contract, increasing the amplitude and the frequency of contraction (Fig. 1). All ten investigated sphincter preparations reacted in the same way but to a various degree.

The effect of tetrodotoxin was dose-dependent and the response diminished and changed pattern after a few minutes while tetrodotoxin still was left in the organ bath (Fig. 1). Tachyphylaxis to repeated doses also developed but might at least partly be ascribed to the property of the preparation to show decreased reactivity to pharmacological agents together with decreased spontaneous activity with time (Persson, 1971). The spontaneous activity of the sphincter usually disappeared about 3 h after the mounting of the sphincter in the bath. Then tetrodotoxin (0·1–1 μg/ml) was able to transiently induce the activity again. Tetrodotoxin also potentiated the excitatory effect of noradrenaline and acetylcholine on the sphincter. This effect was most defined on the non-active sphincters and when tetrodotoxin was given in threshold active doses before addition of noradrenaline and acetylcholine (Fig. 2). Atropine and phenoxybenazmine in effective anti-acetylcholine and α-adrenoceptor

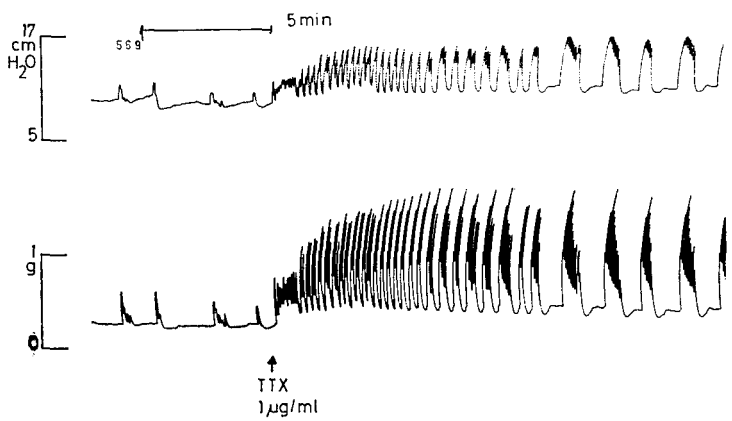

FIG. 1. Effect of tetrodotoxin (TTX) on the isolated sphincter of Oddi mounted longitudinally and perfused at a constant rate (3 ml/h). *Upper curve:* Resistance to flow thrugh the sphincter recorded as pressure changes in the perfusion catheter. *Lower curve:* Longitudinal tension changes. Tetrodotoxin is added to the bathing solution.

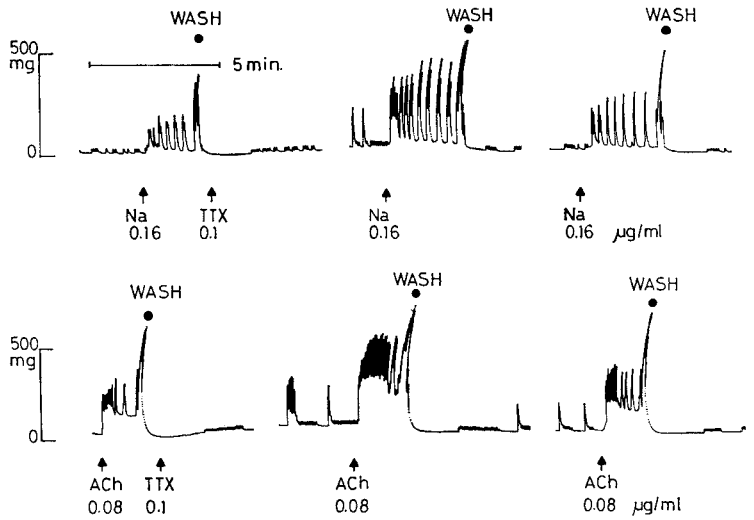

FIG. 2. *Upper curve:* Longitudinal activity of the isolated sphincter. Tetrodotoxin (TTX) 0·1 µg/ml potentiates the effect of noradrenaline (Na). *Lower curve:* Longitudinal activity of the isolated sphincter. Tetrodotoxin (TTX) 0·1 µg/ml potentiates the effect of acetylcholine (ACh).

blocking concentrations (0·1 µg/ml) did not prevent the excitatory action of tetrodotoxin. Atropine and phenoxybenazmine was left in contact with the sphincter for 10–40 min before tetrodotoxin was added to the bath.

Four other different smooth muscle preparations with similar myogenic spontaneous activity to the sphincter were included in the study: cat duodenum mounted as a 3 cm long whole piece (10 preparations) or as strips of the longitudinal muscle (5 preparations), rabbit jejunum (10 preparations) and uterus (6 preparations) from oestrogen-treated rats. In none of these isolated organs did tetrodotoxin (0·1–1 µg/ml) increase the spontaneous activity or affect the response to acetylcholine or noradrenaline.

Results from isolated smooth organs suggest that the sphincter has a unique tetrodotoxin sensitive property. Possibly the potentiation of cardiovascular response to noradrenaline and tyramine by tetrodotoxin as reported by Bell (1968) might be a similar effect. It has been stressed by Carter (1969), who showed that part of splanchnic nerves was resistant to tetrodotoxin, that tetrodotoxin should not be used blindly as a selective nerve-inhibiting agent in experiments with innervated smooth muscle organs. The effect of tetrodotoxin on the sphincter of Oddi is another effect of the agent that should be considered when utilizing it as a chemically "denervating" agent.

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