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DIFFERENTIAL EFFECTS OF TETRODOTOXIN ON SYMPATHOMIMETIC ACTIONS OF NICOTINE AND TYRAMINE*

BY

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It is well known that nicotinic stimulants, such as acetylcholine and nicotine, are capable of exerting a sympathomimetic effect in adrenergically innervated tissues. In some organs this effect has been correlated with the release of catecholamines and it has been found that the action of acetylcholine and nicotine can be abolished by procedures which interfere with the functional integrity of the nervous supply, such as administration of reserpine and chronic denervation (see Ferry, 1966). Burn and his co-workers have proposed that the release of noradrenaline in response to sympathetic nerve stimulation is mediated by an intra-axonal cholinergic mechanism (Burn & Rand, 1959, 1965; Burn, 1966), and that the nicotinic stimulants exert their sympathomimetic effect by mimicking the action of endogenous acetylcholine. On the other hand, since acetylcholine and nicotine can initiate antidromic vollevs in sympathetic fibres (Ferry, 1963; Cabrera & Torrance, 1964; Cabrera, Torrance & Viveros, 1966), the sympathomimetic action of these drugs may be attributable to stimulation of adrenergic fibres.

Tetrodotoxin has been reported to block axonal conduction in various nerve-smooth muscle preparations (Osa & Toida, 1966; Tomita, 1966; Bülbring & Tomita, 1967; Gershon, 1967; Hashimoto, Holman & McLean, 1967). It was therefore of interest to examine the effects of this toxin on the sympathomimetic actions of nicotinic stimulants. The present paper compares, in four adrenergically innervated tissues, the effects of tetrodotoxin on the responses to nicotine with those on the responses to tyramine, which releases noradrenaline from adrenergic nerve endings (Fleckenstein & Stöckle, 1955; Burn & Rand, 1958; Muscholl, 1966).

METHODS

The preparations used were the isolated taenia coli-periarterial nerve preparation of the guinea-pig, the rat isolated vas deferens, the isolated perfused ear artery of the rabbit and the guinea-pig isolated atria.

Guinea-pig taenia coli. A section of taenia coli approximately 3 cm long was isolated together with its arterial supply and mounted in modified Krebs solution (Huković, 1961) at 37° C. The blood vessels were clamped over platinum electrodes for stimulation of the periarterial nerves.

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Rat vas deferens. The vas deferens was dissected free from its extrinsic nerve supply and mesenteric investment and mounted in modified Krebs solution at 37° C. The urethral end of the vas deferens was stimulated transmurally by means of platinum ring electrodes.

Rabbit ear artery. Segments 3–6 cm long of the central artery of the rabbit ear were cannulated at the proximal end; they were suspended in McEwen's solution (McEwen, 1956) and perfused with the same solution (de la Lande & Rand, 1965). The periarterial nerves were stimulated with platinum ring electrodes applied to the proximal end of the segment.

Guinea-pig atria. The two atria were isolated and mounted as a pair in McEwen's solution at 26° C. They were stimulated transmurally by means of two platinum strip electrodes placed in the bath at either side of the tissue.

A Grass S5 stimulator was used to deliver rectangular pulses of 0.2–1 msec duration and supramaximal strength. Tension was recorded with either a frontal point writing lever on a smoked drum or a Grass FTO3 strain gauge and a Grass Model 5 polygraph. Perfusion pressure of the arterial segments was recorded with a Condon manometer writing on a smoked drum or with a Statham P23BC pressure transducer and a Grass polygraph.

Preparations of taenia coli and atria were pretreated with hyoscine (10^{-6} g/ml.) to abolish the effects of stimulation of cholinergic nerves. In the other preparations hyoscine pretreatment was used initially, but was found to be unnecessary.

Drugs. These were: cocaine hydrochloride, nicotine hydrogen tartrate, noradrenaline hydrogen tartrate, tyramine hydrochloride and tetrodotoxin (citrate-free). All drugs were dissolved in distilled water and administered in volumes of not more than 0.1 ml. Ascorbic acid (10^{-6} g/ml.) was added to solutions of noradrenaline and tyramine to prevent oxidation. The fluids in which the drugs were dissolved had no effects on the preparations. In experiments on the perfused artery, drugs were injected into the fluid perfusing the tissue. In the other experiments the drugs were added directly to the organ bath, the concentration given being the final concentration in the bath fluid. All weights refer to the salts of the drugs.

RESULTS

Guinea-pig taenia coli. Stimulation of the periarterial nerves (20–40 pulses/sec, 1 msec pulse duration for 10 sec) noradrenaline (10^{-6} g/ml.) and nicotine ($2–4 \times 10^{-6}$ g/ml.) caused reproducible relaxations of the muscle in six preparations. After addition of tetrodotoxin ($10^{-7}–5 \times 10^{-7}$ g/ml.) to the bath, the responses to nerve stimulation and nicotine were abolished, while the response to noradrenaline was unaffected (Fig. 1). Tyramine in concentrations up to 10^{-5} g/ml. had no sympathomimetic effect on the taenia coli.

Rat vas deferens. Transmural nerve stimulation (20–50 pulses/sec, 0.2 msec pulse duration for 10 sec), noradrenaline ($2–5 \times 10^{-6}$ g/ml.) and tyramine (10^{-5} g/ml.) caused reproducible contractions of the vas deferens. In one out of five experiments nicotine (10^{-5} g/ml.) caused reproducible contractions of comparable amplitude with those induced by other means of excitation (Fig. 2). In the remaining four experiments no consistent responses to nicotine in concentrations up to 10^{-4} g/ml. were obtained. Tetrodotoxin ($2–5 \times 10^{-7}$ g/ml.) abolished the responses to nerve stimulation and to nicotine, but did not significantly affect the responses to tyramine and noradrenaline (Fig. 2). As the response to tyramine was abolished by cocaine (10^{-5} g/ml.), the action of tyramine was probably caused by release of noradrenaline and not by a direct effect on the muscle (Muscholl, 1966).

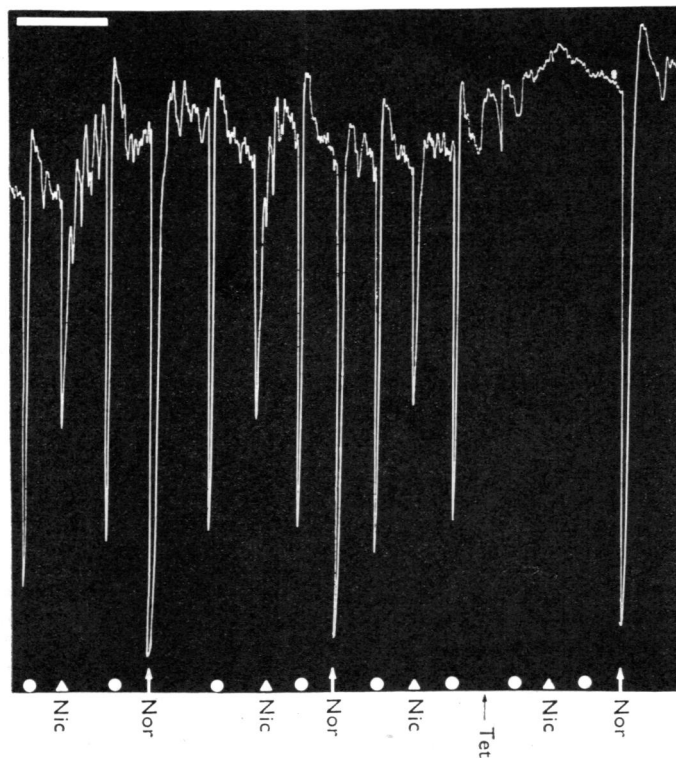

Fig. 1. Responses of the guinea-pig taenia coli to stimulation of the periarterial nerves, nicotine and noradrenaline. White dots, stimulation of periarterial nerves (40 pulses/sec, 1 msec pulse duration, for 10 sec); Nic, nicotine (4×10^{-6} g/ml.); Nor, noradrenaline (10^{-6} g/ml.). At Tet, tetrodotoxin (10^{-7} g/ml.) was added to the bath. Time marker, 10 min.

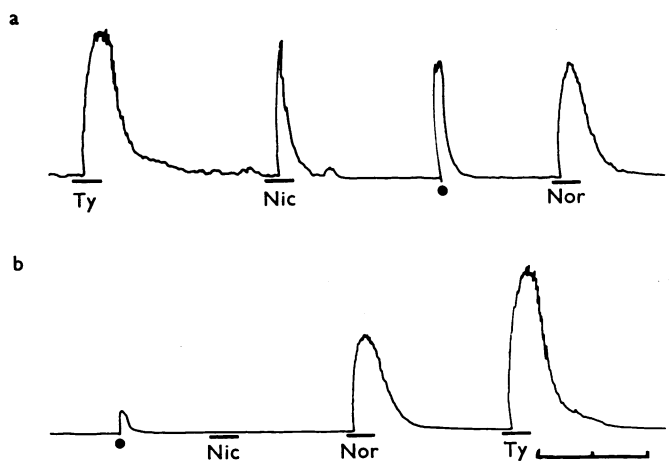

Fig. 2. Responses of the rat vas deferens to transmural nerve stimulation, tyramine, noradrenaline and nicotine, (a) before and (b) after addition to the bath of tetrodotoxin (5×10^{-7} g/ml.). Black dots, transmural nerve stimulation (40 pulses/sec, 0.2 msec pulse duration, for 10 sec); Ty, tyramine (10^{-5} g/ml.); Nor, noradrenaline (2×10^{-6} g/ml.); Nic, nicotine (10^{-5} g/ml.). Time marker, 1 min intervals.

Rabbit ear artery. Stimulation of the periarterial nerves (20–40 pulses/sec, 1 msec pulse duration for 10 sec), noradrenaline (5×10^{-9}–2×10^{-8} g), nicotine (5×10^{-5}–10^{-4} g) and tyramine (2×10^{-5}–2×10^{-4} g) caused reproducible increases in perfusion pressure in six preparations. In a further three preparations no consistent responses to nicotine were obtained, although the responses to other forms of stimulation were normal. Tetrodotoxin (10^{-7}–5×10^{-7} g/ml. external bathing medium) abolished the responses to nerve stimulation and nicotine (Fig. 3), while the response to noradrenaline was unaffected or reduced only slightly. The indirect phase of the response to tyramine (Farmer, 1966) was unaffected or potentiated by tetrodotoxin (Fig. 3), but was abolished by cocaine (10^{-5} g/ml.).

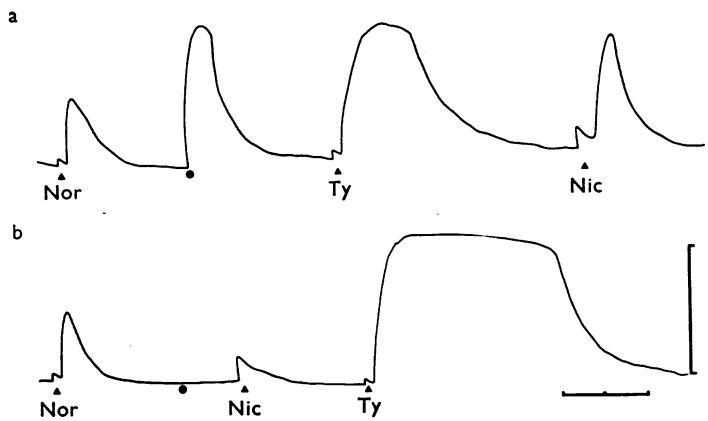

Fig. 3. Perfusion pressure of the isolated ear artery of the rabbit in response to stimulation of the periarterial nerves and intraluminal injections of noradrenaline, nicotine and tyramine, (a) before and (b) after addition of tetrodotoxin (5×10^{-7} g/ml.) to the external medium. Black dots, stimulation of the periarterial nerves (20 pulses/sec, 1 msec pulse duration, for 10 sec); Nor, noradrenaline (10^{-8} g); Nic, nicotine (5×10^{-5} g); Ty, tyramine (4×10^{-5} g). Calibrations, 60 mm of mercury and 1 min intervals. The small rises in pressure preceding drug responses are injection artefacts.

Guinea-pig atria. Transmural stimulation of the sympathetic nerves (2–5 pulses/sec, 0.2–0.4 msec pulse duration for 15–30 sec), noradrenaline (5×10^{-8} g/ml.), nicotine (5×10^{-6} g/ml.) and tyramine (10^{-5} g/ml.) produced positive inotropic and chronotropic responses in four preparations of the spontaneously beating atria. Tetrodotoxin (5×10^{-7} g/ml.) abolished both the inotropic and chronotropic effects of nerve stimulation and nicotine (Fig. 4, Table 1). The inotropic effects of tyramine and noradrenaline were either unaffected or potentiated in the presence of tetrodotoxin (Fig. 4), while the chronotropic effect of noradrenaline was consistently potentiated and that of tyramine somewhat reduced or little affected (Table 1). Both the inotropic and chronotropic responses to tyramine were abolished by cocaine (10^{-5} g/ml.).

DISCUSSION

In a variety of adrenergically innervated tissues the response to sympathetic nerve stimulation and the sympathomimetic effects of nicotine were abolished by low concen-

trations of tetrodotoxin. In contrast, tetrodotoxin did not markedly reduce the response of these tissues to tyramine or to noradrenaline, an observation which indicates that neither the capacity of the nerves to release transmitter nor the sensitivity of the muscle was impaired by the toxin.

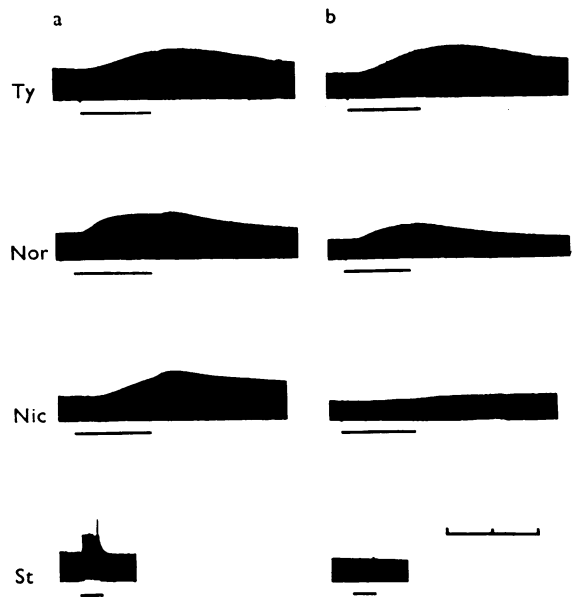

Fig. 4. Inotropic responses of the guinea-pig isolated atria to transmural nerve stimulation, tyramine, noradrenaline and nicotine, (a) before and (b) after addition to the bath of tetrodotoxin (5×10^{-7} g/ml.). Black bars represent periods of drug contact and stimulation. St, transmural nerve stimulation (4 pulses/sec, 0.2 msec duration, for 30 sec); Ty, tyramine (10^{-5} g/ml.); Nor, noradrenaline (5×10^{-8} g/ml.); Nic, nicotine (5×10^{-5} g/ml.). Time marker, 1 min intervals.

TABLE 1

INCREASE IN RATE (BEATS/MIN) OF FOUR PREPARATIONS OF THE GUINEA-PIG ATRIA IN RESPONSE TO TRANSMURAL STIMULATION

(4 pulses/sec, 0·2 msec duration, for 15–30 sec), nicotine (5×10^{-6} g/ml.), noradrenaline (5×10^{-8} g/ml.) and tyramine (10^{-5} g/ml.), (a) before and (b) after tetrodotoxin (5×10^{-7} g/ml.)

Expt.	Transmural stimulation		Nicotine		Noradrenaline		Tyramine	
	a	b	a	b	a	b	a	b
1	40	0	22	0	26	28	22	11
2	50	0	10	0	10	12	18	12
3	43	0	11	0	13	26	10	11
4	—	—	15	3	16	28	12	15

The action of tetrodotoxin in abolishing action potentials in nerve and skeletal muscle fibres has been shown to be the result of a specific block of the rise in Na^+ conductance (Narahashi, Moore & Scott, 1964; Nakamura, Nakajima & Grundfest, 1965; Takata, Moore, Kao & Fuhrman, 1966). In contrast to the relatively non-specific actions of local anaesthetics on ionic conductance (Shanes, Freygang, Grundfest & Amatniek, 1959;

Taylor, 1959; Narahashi, Anderson & Moore, 1967), tetrodotoxin does not affect K^+ conductance. Furthermore, tetrodotoxin does not block action potentials in barnacle and crayfish muscle, where the current responsible for the rising phase of the action potential is carried by Ca^{2+} ions (Hagiwara & Naka, 1965; Hagiwara & Nakajima, 1966; Kao, 1966), and in the plant cell *Nitella*, where this current is carried by Cl^- ions (Kishimoto, 1965, quoted by Kao, 1966). In addition, tetrodotoxin does not block conducted activity in various smooth muscles (Hashimoto *et al.*, 1967; Kuriyama *et al.*, 1966; Tomita, 1966; Bülbring & Tomita, 1967). There is a good deal of evidence to suggest that, in these tissues, at least part of the current responsible for the rising phase of the action potential is carried by Ca^{2+} ions (Bülbring & Kuriyama, 1963; Bennett, 1967). The abolition of the sympathomimetic effect of nicotine by tetrodotoxin therefore suggests that impulse conduction along an axon is involved. Conversely, the fact that tetrodotoxin does not abolish the responses to tyramine indicates that the sympathomimetic action of this drug is independent of an increase in Na^+ conductance.

Two theories exist regarding the mechanism of noradrenaline release from adrenergic axons in response to nerve stimulation. The first, proposed by Burn & Rand in 1959. was recently restated in the following terms (Burn & Rand, 1965; Burn, 1966). The arrival of an action potential at the nerve ending releases acetylcholine from an intra-axonal store. This acetylcholine increases the permeability of the axonal membrane to Ca^{2+} ions, which in turn cause liberation of noradrenaline into the extracellular space. Similarly, the sympathomimetic effect of nicotine is assumed to be due to increased Ca^{2+} influx.

The second theory is that the action potential causes release of noradrenaline from the axon without the intervention of a cholinergic mechanism. Thus, according to this theory, the sympathomimetic effect of nicotine must be the result of some action other than that proposed by the Burn–Rand theory. It has been shown by Ferry (1963), Cabrera & Torrance (1964) and Cabrera *et al.* (1966) that acetylcholine and nicotine, administered by close intra-arterial injection, induce antidromic firing in the sympathetic fibres supplying the spleen, skin and heart of the cat. These observations suggest that nicotine may exert its sympathomimetic effect at least partly by initiation of action potentials in the sympathetic axons.

The results of the present study indicate that a propagated action potential is an essential part of the sympathomimetic effect of nicotine and so favours the interpretation that nicotine is acting on the axonal membrane rather than on an intra-axonal mechanism.

SUMMARY

1. The actions of tetrodotoxin on the sympathomimetic effects of tyramine, nicotine and noradrenaline, and on the response to sympathetic nerve stimulation were examined on the isolated taenia coli/periarterial nerve preparation of the guinea-pig, the rat isolated vas deferens, the isolated perfused ear artery of the rabbit and the guinea-pig isolated atria.

2. Consistent responses to nicotine were always obtained on the taenia coli and atria, in six out of nine preparations of the perfused artery and in only one out of five prepara-

tions of the vas deferens. Tyramine was inactive on the taenia coli but gave consistent responses on the other tissues.

3. Tetrodotoxin (10^{-7}–5×10^{-7} g/ml.) abolished the responses to sympathetic nerve stimulation and to nicotine. In contrast, the responses to tyramine, which releases noradrenaline, and to noradrenaline itself were not markedly reduced, and were potentiated in some preparations.

4. It is concluded that the sympathomimetic action of nicotine, but not of tyramine, is dependent on a propagated action potential. The results favour the theory that the effect of nicotine is the result of excitation of the axonal membrane rather than of an activation of an intra-axonal mechanism as suggested by the Burn–Rand theory.

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