EFFECTS OF SURUGATOXIN ON ADRENERGICALLY INNERVATED TISSUES

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Abstract—Effects of surugatoxin (SGTX), a new ganglionic blocking agent on adrenergically innervated tissues: guinea pig isolated atria and vas deferens were investigated. SGTX (1, 10 μ M) markedly reduced the cardiostimulatory response of the atria to nicotine and also partially the response to 1,1-dimethyl-4-phenylpiperazinium (DMPP) and 5-hydroxytryptamine, without affecting responses to noradrenaline, tyramine and histamine. The contractile response of the vas deferens to nicotine, DMPP and hypogastric nerve stimulation was markedly reduced by SGTX (1, 10 μ M), whereas that to noradrenaline, acetylcholine and transmural stimulation was not affected. These results indicate that SGTX has an antagonistic action on nicotinic receptors in these tissues as well as in sympathetic ganglia and in guinea pig ileum.

In previous studies, we found that surugatoxin (SGTX) isolated from Japanese ivory mollusc, *Babylonia japonica* (1) was a potent antagonist of nicotinic stimulants in the cat superior cervical ganglia and celiac ganglia, rat superior cervical ganglia and guinea pig ileum (2-5). Nicotinic stimulants exert a sympathomimetic effect in adrenergically innervated tissues and this effect has been correlated with the release of catecholamines from the sympathetic nerve terminals (6-12). In the present study, the effect of SGTX on sympathomimetic responses to nicotinic stimulants and to sympathetic nerve stimulation was investigated in adrenergically innervated tissues: guinea pig isolated atria and vas deferens.

MATERIALS AND METHODS

Male guinea pigs weighing 300-400 g were sacrificed by a blow on the head, and the heart or vas deferens was quickly removed. The atria were carefully dissected from adjacent tissues and suspended in a 30 ml organ bath containing Tyrode's solution which was maintained at 30 $\pm$ 1 $^{\circ}$ C and bubbled with a mixture gas of 95% O₂-5% CO₂. The composition of Tyrode's solution was as previously described (2). The atria were attached to a Grass force-displacement transducer, and isometric contractile force (resting tension of approximately 0.5 g) and rate of spontaneous beat were recorded by means of a recticorder (Nihon Kohden, RJG-3024). The increase in responses to agonists is expressed as percent of control response before the addition of agonists.

The vas deferens was suspended in a 10 ml organ bath containing Krebs's solution which was maintained at 37 $\pm$ 1 $^{\circ}$ C and bubbled with a mixture gas of 95% O₂-5% CO₂. The preparation was subjected to a resting tension of 0.5 g and the isometric contraction was

recorded. The composition of the Krebs's solution was the following in (mM): Na^+ 137; K^+ 5.9; Ca^{++} 2.5; Mg^{++} 1.2; Cl^- 134; HCO_3^- 15.5; H_2PO_4^- 1.2; glucose 11.5. In some experiments, vas deferens was removed together with the hypogastric nerve (13). The hypogastric nerve was stimulated with maximal rectangular pulses (2 msec, 25 Hz, maximal voltage) for 5 sec at 3 min intervals by an electric stimulator (Nihon Kohden, MSE-3R). Transmural stimulation was also carried out by submaximal pulses (2 msec, 25 Hz, submaximal voltage) for 5 sec at 3 min intervals (14). The preparation were allowed to equilibrate at least 1 hr before experiments and during this period the bath solution was changed every 20 minutes. SGTX and some antagonists were added at 15–60 min before the addition of agonists. Responses to the agonists obtained in the presence of antagonists are expressed as percent of the preceding control response. Statistical calculation was carried out by means of Student's *t*-test.

The following drugs were used: acetylcholine chloride (Daiichi), carbamylcholine chloride (Sigma), histamine diphosphate (Wako), nicotine tartrate (Wako), 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP, Aldrich), tyramine hydrochloride (Daiichi), 5-hydroxytryptamine (Wako), noradrenaline hydrochloride (Sankyo), hexamethonium chloride (Wako), mecamlamine hydrochloride (Sigma), atropine sulphate (Merck), propranolol hydrochloride (Sumitomo), cocaine hydrochloride (Sankyo), phenoxybenzamine hydrochloride (Tokyo Kasei), reserpine (Daiichi), bretylium tosylate (Tokyo Kasei), hemicholinium-3 (Aldrich), hyoscine bromide (Merck), tetrodotoxin (Sankyo). SGTX was provided by Prof. T. Kosuge, Shizuoka College of Pharmaceutical Sciences. Drugs were dissolved in distilled water and the concentrations refer to the weights of the salts.

RESULTS

Effects on the guinea pig isolated atria

SGTX had little effect on the spontaneously beating atria of guinea pigs at concentrations of less than 10 μM .

Nicotine (30 μM) and DMPP (30 μM) in the presence of hyoscine (0.3 μM) exerted a marked positive inotropic (increase rate, nicotine: $129.2 \pm 13.8\%$, $n=5$, DMPP: $114.5 \pm 9.7\%$, $n=4$) and a slight positive chronotropic (increase rate, nicotine: $22.3 \pm 4.3\%$,

FIG. 1. Effects of SGTX on the positive inotropic and chronotropic responses of guinea pig isolated atrium to nicotine in the presence of hyoscine (0.3 μM). (a) and (b) indicate cardiostimulatory responses to nicotine (Ni, 0.1 mM) in the absence and presence of SGTX (1 μM), respectively.

TABLE 1. Effects of ganglionic blocking agents on the positive inotropic and chronotropic responses of guinea pig isolated atria to nicotine, DMPP (in the presence of hyoscine 0.3 μ M) and 5-hydroxytryptamine

Pretreatment	Dose (μ M)	Increase (%) in response of atria				5-hydroxytryptamine (50 μ M)	
		Nicotine (30 μ M)		DMPP (30 μ M)		Inotropic	Chronotropic
		Inotropic	Chronotropic	Inotropic	Chronotropic		
Mean $\pm$ S.E. (n)							
None		129.2 $\pm$ 13.8(5)	22.3 $\pm$ 4.3(5)	114.5 $\pm$ 9.7(4)	26.1 $\pm$ 3.9(4)	61.8 $\pm$ 12.0(5)	14.7 $\pm$ 3.8(4)
SGTX	1	3.2 $\pm$ 1.1(3)***	2.8 $\pm$ 0.8(3)**				
	10			53.4 $\pm$ 10.2(3)*	9.9 $\pm$ 3.7(3)*	30.3 $\pm$ 5.5(3)*	3.1 $\pm$ 1.0(3)*
Hexamethonium	30	4.2 $\pm$ 0.4(3)***	2.2 $\pm$ 0.8(3)**				
	100			54.7 $\pm$ 12.4(4)*	10.3 $\pm$ 4.1(4)*	60.0 $\pm$ 10.4(3)	11.6 $\pm$ 2.4(3)
Mecamylamine	3	9.0 $\pm$ 0.5(3)***	3.4 $\pm$ 0.6(3)*				
	10			40.0 $\pm$ 5.5(3)**	8.1 $\pm$ 3.1(3)**	38.2 $\pm$ 8.4(3)	6.8 $\pm$ 1.8(3)

Blocking agents were added 15 min prior to the stimulants. Inotropic and chronotropic responses are expressed as % of preceding control response. Significant difference from control response: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

FIG. 2. Effects of SGTX on positive inotropic (a) and chronotropic (b) responses of guinea pig isolated atria to nicotine (30–300 μ M) in the presence of hyoscine (0.3 μ M). (a) and (b) indicate cardiostimulatory responses to nicotine expressed as % of preceding control response, in the absence (●) and presence (▲) of SGTX (1 μ M). Each point represents the mean $\pm$ S.E. of 3–5 experiments. Nicotine (30–300 μ M) was added at 50 min intervals by the single dose method.

$n=5$, DMPP: $26.1 \pm 3.9\%$, $n=4$) responses on the guinea pig isolated atria (Fig. 1a, Table 1). The responses to nicotine and DMPP were reproducible in the preparation washed 3 to 4 times with Tyrode's solution at 15–20 min intervals. SGTX (1 μ M) markedly reduced positive inotropic and chronotropic responses to nicotine (30 μ M) to $3.2 \pm 1.1\%$ and $2.8 \pm 0.8\%$ ($n=3$), respectively (Fig. 1b, Table 1) and the inhibition rapidly disappeared after washout of SGTX. Figure 2 shows the inhibitory effect of SGTX on dose response curves for nicotine (30–300 μ M) and thus SGTX (1 μ M) reduced the cardiostimulatory response to nicotine by approximately 90%. Positive inotropic and chronotropic responses to DMPP (30 μ M) were reduced by SGTX (10 μ M) to $53.4 \pm 10.2\%$ and $9.9 \pm 3.7\%$ ($n=3$), respectively. SGTX (10 μ M) did not affect the cardiostimulatory responses to noradrenaline (0.1 μ M), histamine (10 μ M) and tyramine (30 μ M), whereas responses to 5-hydroxytryptamine (50 μ M) were reduced by 50–70% (Table 1).

The cardiostimulatory responses to nicotine (30 μ M) and DMPP (30 μ M) were significantly reduced by hexamethonium (30, 100 μ M) and mecamylamine (3, 10 μ M). The response to 5-hydroxytryptamine was inhibited by mecamylamine (10 μ M), but not by hexamethonium (100 μ M) (Table 1). The cardiostimulatory responses to nicotine (30 μ M), DMPP (30 μ M) and 5-hydroxytryptamine (50 μ M) were significantly reduced by the pre-treatment of animals with reserpine (5 mg/kg i.p.) or by the addition of bretylium (30 μ M), propranolol (1 μ M) and cocaine (10 μ M).

Effects on guinea pig isolated vas deferens

The reproducible contraction of guinea pig isolated vas deferens was obtained by addition of nicotinic stimulants to the organ bath at 40–50 min intervals. SGTX (1 μ M) reversibly reduced the contractile responses to nicotine (0.1 mM) and DMPP (0.1 mM) by $95.3 \pm 4.2\%$ ($n=3$) and $76.0 \pm 6.7\%$ ($n=3$) respectively (Fig. 3a, Table 2). The contractile response to hypogastric nerve stimulation was markedly reduced by SGTX (10 μ M) although the response to transmural stimulation was unaffected (Fig. 3c). SGTX (10 μ M) enhanced the contractile response to noradrenaline (10 μ M) whereas it had no or little effect on that to acetylcholine (10 μ M) or carbachol (0.1 mM) (Fig. 3b). As shown in Table 2, similar

FIG. 3. Effects of SGTX on the contractile response of guinea pig isolated vas deferens to agonists and to electrical stimulation of sympathetic nerves. (a) and (b) indicate the contractile responses of vas deferens to nicotine (Ni, 0.1 mM), DMPP (D, 0.1 mM), noradrenaline (NA, 10 μ M) and acetylcholine (ACh, 10 μ M) in the absence and presence of SGTX (1, 10 μ M), and (c) indicates that to hypogastric nerve stimulation (HNS) and to transmural stimulation (TS) in hypogastric nerve-vas deferens preparation. The time of contact with agonists was 30 sec and the interval between was 30 min. SGTX was added 15 min prior to addition of the agonists.

results were obtained with hexamethonium (10 μ M) and mecamylamine (3 μ M). The contractile response to nicotine (0.1 mM) and DMPP (0.1 mM) was inhibited by bretylium (30 μ M), phenoxybenzamine (10 μ M), cocaine (3 μ M), atropine (1 μ M) and hemicholinium-3 (0.1 mM) but not by tetrodotoxin (3 μ M). The response to nicotine was more sensitive to the blockade of phenoxybenzamine, cocaine, atropine and hemicholinium-3.

DISCUSSION

Effects of SGTX on adrenergically innervated tissues: guinea pig isolated atria and

TABLE 2. Effects of ganglionic, adrenergic and cholinergic blocking agents on the contractile responses of guinea pig isolated vas deferens to nicotine and DMPP

Pretreatment	Dose	Inhibition rate (%)	
	(μ M)	Nicotine (0.1 mM)	DMPP (0.1 mM)
	Mean $\pm$ S.E. (n)		
SGTX	1	95.3 $\pm$ 4.2 (3)	76.0 $\pm$ 6.7 (3)
Hexamethonium	10	97.4 (2)	78.9 (2)
Mecamylamine	3	98.2 (2)	69.0 (2)
Bretylium	30	68.5 (2)	69.0 (2)
Phenoxybenzamine	10	100.0 (2)	87.5 (2)
Cocaine	3	100.0 (2)	70.0 (2)
Atropine	1	81.6 (2)	49.6 (2)
Hemicholinium-3	100	96.5 (2)	64.8 (2)

All blocking agents except phenoxybenzamine were added 15 min prior to the stimulants and phenoxybenzamine 60 min prior to them.

vas deferens were investigated. SGTX significantly reduced the sympathomimetic response of these tissues to nicotinic stimulants without affecting responses to noradrenaline, acetylcholine and histamine. The results indicate that SGTX has an antagonistic action on nicotinic receptors in these tissues as well as in sympathetic ganglia and guinea pig ileum.

The cardiostimulatory responses to nicotine and DMPP were reduced by adrenergic blocking agents: reserpine, bretylium and propranolol, indicating that nicotinic stimulants exert their cardiostimulatory responses on guinea pig isolated atria by the release of catecholamines from postganglionic nerves (6-8, 11, 15). The release of noradrenaline by nicotine was dependent on the presence of external calcium ion (11) and was possibly produced by an increase of permeability of calcium at the nerve terminals (12, 15). SGTX did not affect the cardiostimulatory response to tyramine which is reportedly calcium-independent (11). In addition SGTX markedly reduced DMPP- and acetylcholine-stimulated uptake of ^{45}Ca by perfused bovine adrenal glands (16). Therefore, it may be considered that SGTX blocks the nicotinic receptors in sympathetic nerve terminals as well as in the ganglia, and induces an inhibition of calcium ion influx resulting in the reduced release of noradrenaline. The failure of SGTX to abolish the sympathomimetic response to DMPP might be explained on the basis that a part of its action on guinea pig atria was similar to the action of tyramine (8, 15).

The cardiostimulatory response to 5-hydroxytryptamine was partially antagonized by high concentrations of SGTX and mecamylamine, but not by hexamethonium. In this respect SGTX differs from hexamethonium and more closely resembles mecamylamine. In the present experiments, the cardiostimulatory response was reduced by reserpine, bretylium and propranolol, suggesting an indirect action involved in the release of noradrenaline (17). It has been reported that two types of 5-hydroxytryptamine receptors are located in the muscle site (D) and in nerve site (M) of ileal smooth muscle (18). The blocking effect of SGTX on the M-receptor in guinea pig ileum and rat superior cervical ganglia has been reported (2, 3). Therefore, a similar effect of SGTX on the M-receptor may be re-

sponsible for the inhibitory effect on guinea pig isolated atria.

Contractile responses of guinea pig isolated vas deferens to nicotine and DMPP as well as to hypogastric nerve stimulation were reduced by SGTX. Adrenergic and cholinergic mechanisms appear to be involved in responses of the vas deferens to nicotinic stimulants since these responses were reduced by both adrenergic and cholinergic blocking agents, as is the case in the response to hypogastric nerve stimulation (19). It has been suggested that there are peripheral ganglionic synapses in sympathetic innervation to the vas deferens (15, 20). Thus it is likely that SGTX may act on the ganglionic sites as well as on the sympathetic nerve terminals in guinea pig vas deferens.

In addition, a high concentration of SGTX potentiated the contractile response of the vas deferens to noradrenaline. Pressor response to adrenaline in cats was also enhanced by pretreatment with SGTX (2). Similarly, some ganglionic blocking agents potentiated the pressor responses to catecholamines (21–23). This effect was reported to be initiated by the direct action of ganglionic blocking agents on effector cells (22, 23). Therefore, the potentiative effect of SGTX may be due to a direct action on effector cells, this action being independent on ganglionic blockade.

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